

FINAL REPORT

19990198

**SASKATCHEWAN DERIVED FEEDSTUFFS
FOR AQUACULTURE**

October, 2004

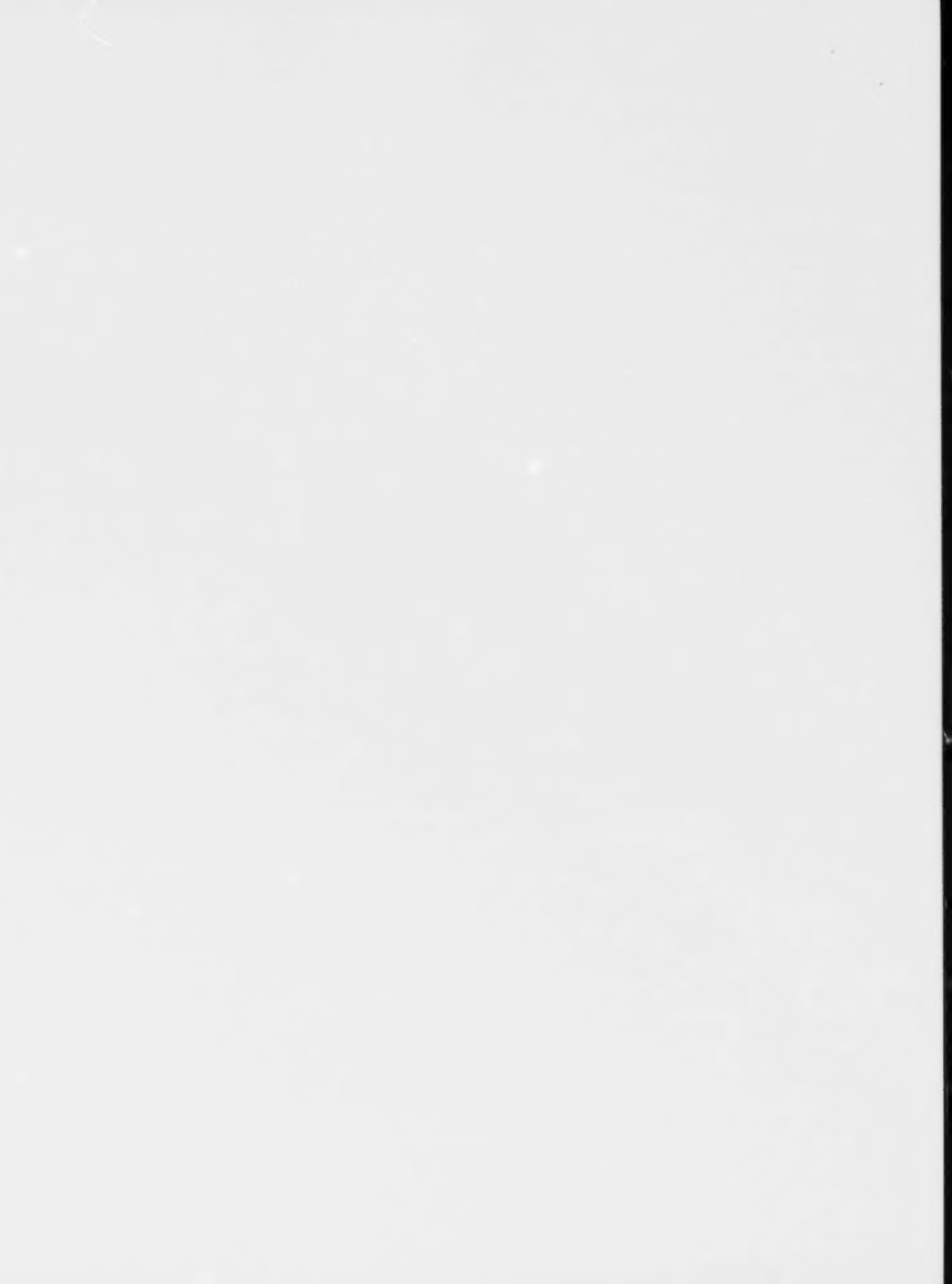
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Saskatchewan Derived Feedstuffs for Aquaculture

Final Report

19990198

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1) Summary

The final year of support of the Prairie Feed Resource Centre's aquaculture feed development program saw the completion of testing flax, pea protein concentrate and canola protein concentrate as feed ingredients for rainbow trout and the initiation of testing flax, canola protein concentrate and pea protein concentrate as feed ingredients for another important aquaculture species: tilapia. Based on the results of research over the last 4 years, commercial activity to produce pea protein concentrate, and dehulled flax has been initiated in Saskatchewan and the use of canola protein concentrates in aquaculture diets has been supported. International marketing relationships have been developed with the salmon industry in Chile through Fundacion Chile, a Chilean industry development agency. We have continued to collaborate extensively with industrial partners including EWOS Canada Ltd. (our major industrial partner), MCN Bioproducts Inc., (Manufacturers of Canola Protein Concentrate), Werner Agra Ltd. and Oleet Processing Ltd. (manufacturers of LinPro and ExtraPro) and JEFO Nutrition (manufacturers of a protease with potential in aquaculture diets) and Parrheim Foods (manufacturers of pea protein concentrate).

We have also developed new relationships with Greenview Aquafarms, a major tilapia producer in Calgary AB, and Alberta Agriculture for the development of feed ingredients for the tilapia industry in Western Canada. We continue to be funded by the Saskatchewan Flax Development Commission. We continue to work towards commercialization of flax, canola and peas as aquafeed ingredients, with three community groups interested in value added processing. Lastly, we recently received funding through AquaNet, a Network Centre of Excellence in collaboration with MCN Bioproducts to test Saskatchewan feed ingredients as a means to reduce PCBs and dioxins and other organochlorine compounds in farmed fish.

This project has moved from conception to research to commercialization in the last 4 years. The potential value added impact of this project is approximately \$160 million annually from canola, pea and flax products. These achievements demonstrate that the aquaculture feeds program at the University of Saskatchewan has and is continuing to increase value-added processing of feeds in Saskatchewan and has provided technical marketing support for these feed ingredients to aquaculture feed manufacturers around the world.

2) Technical Report

2.1) Background and objectives

The requirement for non-marine sources of protein and oil in the aquafeed industry has grown even stronger this past year. Fish meal and oil prices rose significantly in 2003 and adversely affected the profitability and sustainability of the industry. Saskatchewan crops offer to the aquafeed industry, unique protein and oil sources particularly flax (oil) and peas and canola (protein, oil). However, these crops will require significant further processing to meet this potential including dehulling and extrusion of flax and canola seed to remove soluble fibre and increase oil digestibility and production of protein concentrates of peas and canola to produce a concentrated protein source that meets the requirement for nutrient dense feed ingredients demanded by aquafeed producers.

Project Objectives

- 1) To assess the value of Saskatchewan crops as feed ingredients for aquaculture diets.
- 2) To develop processing methods to enhance the nutrient value of Saskatchewan crops for the aquafeed industry
- 3) To create links between, Saskatchewan producer groups and processors with the international aquafeed industry.
- 4) To promote Saskatchewan grown feed ingredients to the international aquafeed industry.

2.2) Project activities, achievements, and papers written

Development of Prairie-Based Feed Ingredients for Aquaculture Diets

Funding

- AFIF \$50,000 per year for 4 years (2000-2004).
- EWOS Canada \$60,000 per year for 4 years from EWOS Canada Ltd. (2000-2004).
- Saskatchewan Flax Development Commission \$12,000 per year for 3 years (2001-2004). Project title: Development of a replacement for fishmeal and fish oil using dehulled flax and protein concentrates produced from Western Canadian feed ingredients.
- AquaNet/MCN Bioproducts \$75,880 over 2 years (2004-2006). Project title: Replacement of fish meal and fish oil with canola protein concentrate and dehulled flax in diets fed to rainbow trout (*Oncorhynchus mykiss*).

Background and Project Status

- This year, we have performed 6 studies on the nutritional properties of Saskatchewan feed ingredients concentrating on long-term growth studies of rainbow trout and tilapia fed processed pea, flax and canola products. Full reports of these studies can be found in Appendices I-V at the end of this report. A summary of these reports can be found below.

Appendix I The Effect of Inclusion of a Protease on the Nutrient Digestibility of Dehulled Flaxseed in Rainbow Trout

This experiment was performed to complete our studies on protease addition to flax:pea (Linpro) and canola:pea (Extrapro) coextruded products. The experiment demonstrated that protease addition to diets containing dehulled flax had no effect on protein digestibility. This data was included in a manuscript submitted to Animal Feed Science and Technology. A pre-print of the article is included as **Appendix VI**.

Appendix II Growth performance of rainbow trout (*Oncorhynchus mykiss*) fed CPC canola protein concentrate as a replacement for fish meal.

This study examined the effect of replacing 38 or 76% of the fish meal in a commercial trout diet with canola protein concentrate on fish growth and feed efficiency. Replacing 38% or 76% of the fishmeal in commercial type complex diets with canola protein content did not affect feed intake. Replacement of 38% of fishmeal replacement with CPC had no significant effect on weight gain, however, replacement of 76% of fishmeal with CPC reduced weight gain during the first 4 weeks but no statistically significant differences were obtained in weight gain at 8 and 12 weeks of feeding. Feed conversion rates and protein efficiency ratios were superior for fish on the control diet and these differences were highest at 4 weeks of feeding and decreased at 8 and 12 weeks of feeding. These results suggest that replacement of up to 76% of fishmeal with CPC in commercial salmonid diets is feasible depending on the relative costs of fishmeal and CPC.

Appendix III Effect of processing on the digestibility of Saskatchewan feed ingredients to Nile tilapia (*Oreochromis niloticus*).

These studies examined the effect of feeding raw ingredients (pea, canola meal, or flax) compared to processed ingredients (pea protein concentrate, canola protein concentrate or dehulled flax) in diets fed to tilapia. Energy and dry matter digestibility was significantly improved by processing, however, protein digestibility was not significantly improved by processing. These results suggest that processing improves the nutritional value of pea, canola meal and flax in tilapia diets.

Appendix IV Replacement of fish meal using a complex vs. simple mixture of plant proteins in Nile Tilapia.

This study examined the effect of replacing fish meal with either 2 plant protein ingredients (soybean meal and corn gluten meal; simple diets) or 5 plant protein ingredients (soybean meal, corn gluten meal, pea protein concentrate, canola protein concentrate and dehulled flax; complex diets). A control diet containing 100% protein from fish meal served as a control. We replaced 33.3, 66.7 or 100% of the fish meal with either the simple or complex mixtures of plant protein giving a total of 7 diets. Replacement of 100% of the fishmeal with simple plant proteins resulted in a significant decrease in growth, feed efficiency and intestinal villus length compared to 100% replacement with complex plant proteins. At the 33.3 and 66.7% fishmeal replacement levels, no significant differences were seen in growth performance. However, villus length was significantly decreased at the 66.7% fishmeal replacement level in the simple diets but not in the complex diets. The results suggest that the use of Saskatchewan feed ingredients like pea protein concentrate, canola protein concentrate and dehulled flax in tilapia diets will allow a greater replacement of fishmeal than the use of simple diets currently used by the industry.

Appendix V Effect of replacing fish meal with simple (canola protein concentrate or a mixture of soybean meal and corn gluten meal) or complex (a mixture of canola protein concentrate, soybean meal and corn gluten meal) mixtures of plant proteins in diets fed to Nile Tilapia.

Appendix VI Drew, M.D., Racz, V.J., Gauthier, R., Thiessen, D.L., 2004. Effect of adding protease to coextruded flax:pea or canola:pea products on nutrient digestibility and growth performance of rainbow trout (*Oncorhynchus mykiss*). Animal Feed Science and Technology (in review)

Appendix VII Thiessen, D.L., Maenz, D.D., Newkirk, R.W., Classen, H.L. and Drew, M.D., 2004. Replacement of fish meal by canola protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition (in press)

Indications for Further Development

The use of complex diets for aquaculture species appears to be a significant advantage in replacing fish meal in aquafeeds. This strategy will require commercial availability of plant proteins with a high level of crude protein like pea and canola protein concentrates. Further research on the use of complex diets in aquaculture is required to assess their effect on growth, intestinal morphology, immune function and the level of organochlorines like PCBs and dioxins in fish products. The production of aquafeed ingredients from Saskatchewan crops is rapidly developing and will require research in these areas to support marketing in Canada and the world. The Prairie Aquaculture Research Centre will continue to provide support and leadership in these areas.

2.3) Information on performance indicators in Appendix A

2.3.1) Development of a feed program that will meet the needs of Saskatchewan feed producers.

This has clearly been achieved. The PFRC has facilitated economic ties between producer groups (Saskatchewan Flax Development Commission, Saskatchewan Pulse Growers), feed processors (MCN BioProducts, Werner Agra, MCN BioProducts, Parrheim Foods) and end users (EWOS Canada Ltd.). Based on research performed during the last 4 years and the presentation of these results in Saskatchewan and abroad, commercial production of pea protein concentrate (Northwest Terminal, Unity SK), and dehulled flax (Carlton Trail Pelleting, Kelliher SK) will commence in the next 18 months. Furthermore, market development of canola protein concentrate in Chile, the largest producer of salmon in the world, has been facilitated by research and technical presentations by Murray Drew and Vern Racz in the last year.

2.3.2 Published Papers and Abstracts

Thiessen, D.L., Maenz, D.D., Newkirk, R.W., Classen, H.L. and Drew, M.D., 2004. Replacement of fish meal by canola protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition (in press)

Drew, M.D., Racz, V.J., Gauthier, R., Thiessen, D.L., 2004. Effect of adding protease to coextruded flax:pea or canola:pea products on nutrient digestibility and growth performance of rainbow trout (*Oncorhynchus mykiss*). *Animal Feed Science and Technology* (in review)

T. Borgeson, D.L. Thiessen, V. J. Racz, and M.D. Drew. 2004, Digestibility of Western Canadian Feed Ingredients by Tilapia (*Oreochromis niloticus*). *J. Anim. Sci.* 82(Suppl. 1.):917.

Published as article in *The Western Producer* December 12, 2003. Better feed key to bigger market share. Karen Brier.

Published as article in *Northern Aquaculture*, January 2004. Prairie researchers look to peas as possible fish meal replacement. Quentin Dodd.

2.3.3 Presentations

Processing opportunities in aquafeeds. Processing for Profit: A Conference for Western Canadian Pulse Processors. December 3, 2003. Saskatoon SK.

Prairie Feed Resource Centre Feed Day. Development of value-added proteins for aquaculture. December 18, 2003. Saskatoon SK.

Fundacion Chile. Seminario Internacional Agricultura y Salmonicultura. Puerto Varas, Chile. October 2, 2003. Presentation. The use of vegetable ingredients in aquaculture: The Canadian experience.

Fundacion Chile. Seminario Internacional Agricultura y Salmonicultura. Puerto Varas, Chile. October 2, 2003. Presentation: Economics of replacing fish meal and oil in aquaculture diets.

Salmofood: Castro, Chile. The use of Saskatchewan Feed ingredients in Salmofood aquaculture diet formulations. October 3, 2003.

Value-added innovation; Pulse Crop Growers, Crop Production Week, January 2004, Saskatoon SK.

Indonesian Feed Industry Tour. Prairie Feed Resource Centre. Sept. 10, 2003. Presentation. Alternatives for antibiotics in animal feeds.

Prairie feeds for Chilean aquaculture. Puerot Monte and Santiago Chile, December 2-9, 2003.

Changes and potential value added processing in Western Canadian agriculture. Codrico Corp. Rotterdam NL. April 4, 2004.

2.3.4) Facilitate evaluation and development of new alternative feed stuffs derived from Saskatchewan feeds.

- a) With the testing of flax, pea and canola products (including protein concentrates, dehulled flax, coextruded protein concentrates and flax) as aquafeed ingredients, this milestone has clearly been achieved.
- b) Over the last 4 years, the project has allowed the development of a process of innovation that has taken research to the commercialization phase. This has rapidly developed and in this last report, is showing its potential. The concentration on pea protein concentrate, canola protein concentrates and flax products has been done in consultation with producer groups and the aquaculture industry.

The production of pea protein concentrate for aquaculture will lead to the processing of 160,000 tonnes of peas at one site (Northwest Terminal, Unity SK) for an estimated \$38 million yearly activity. Interest has also been shown by an ethanol facility to utilize the pea starch produced as a coproduct. Canola protein concentrate developed by MCN Bioproducts Ltd. has a potential marked value of \$42 million in the aquaculture industry and this potential has been aided by the research done on this project in the last 4 years. Dehulling of flaxseed as a source of linolenic acid for aquaculture has a potential value of \$60-80 million in yearly activity. The total impact of these 3 products is approximately \$160 million in new feed products, processes and technology. The impact of aquafeed research on value added activity will continue in the foreseeable future. The challenge in the future will be to maintain adequate research activity to realize the full potential of these projects.

2.3.5) Develop infrastructure and personnel necessary to provide leadership with in the aquafeed industry on sustained basis.

- **Infrastructure**

- The Prairie Aquaculture Research Centre has been expanded to 66 culture tanks for the testing of feed ingredients in salmonid fish.
- Analytical capabilities for feed and biological samples have been increased to include fatty acid analysis by gas chromatography and analysis of non-starch polysaccharides.
- A new water heater has been purchased and installed making the culture of warm-water fish species like tilapia possible.

- **Personnel**

- Vern Racz, M.Sc., Director
- Murray Drew, Ph.D. Research Director. 50% of time spent on project work.
- We currently have two graduate students (Debbie Thiessen, Ph.D. candidate and Tracy Borgeson M.Sc. candidate) nearing the completion of their studies. Graduation for both students is expected in the Fall of 2004. Debbie Thiessen has been hired by MCN Bioproducts Inc. as an aquaculture nutritionist.
- A new Ph.D. student, Ayoleke Ogunkoya, from Nigeria will begin studies at the University of Saskatchewan this fall.

4) Equipment

Not Applicable

5) Project Photos

Not applicable

6) Innovation Fund Acknowledgement

We have acknowledged the financial contribution of AFIF in all presentations and publications during the last year.

7) Project Public Information Materials

We have developed a one-page handout for public tours of the Prairie Aquaculture research Centre.

Appendix I

PARC Experimental Log Number:

PARC 0307

Experimental Title:

The Effect of Inclusion of a Protease on the Nutrient Digestibility of Dehulled Flaxseed in Rainbow Trout.

Researchers:

M. Drew, D. Thiessen

Objective:

To determine the effect of a commercial protease enzyme on the digestibility of dehulled flaxseed protein, lipid, energy and dry matter in rainbow trout.

Materials and Method:

The experimental diets for this experiment consisted of the following:

- Diet 1 - Dehulled Flaxseed
- Diet 2 - Dehulled Flaxseed with protease
- Diet 3 - Reference diet
- Diet 4 - Reference diet with protease

Fish Management

Rainbow trout were maintained in 120 L tanks that were part of a recirculating system using biological filtration. Water temperature was maintained at $15 \pm 1^{\circ}\text{C}$. Dissolved oxygen, nitrate, nitrite, ammonia and pH were monitored regularly. Photoperiod was a 14 h light/10 h dark cycle. Ten (436.5g; average weight) fish per tank were utilized with four replicates per treatment. The fish were maintained in accordance with the guidelines of the Canadian Council on Animal Care (CCAC 1984).

Digestibility Determination

Dehulled flaxseed was acquired from the Prairie Agricultural Machinery Institute (Humboldt, SK). The dehulled flaxseed was autoclaved (121°C , 19 psi, 20 min) and ground

(Retsch GM200 mill; 4500 rpm for 10 sec, 6000 rpm for 10 sec) prior to inclusion into the diets. The protease enzyme had an inclusion rate of 0.25 g per 1.00 kg of diet.

Apparent digestibility coefficients (ADC, %) were measured using an indirect method with diets containing 1% celite as a nonabsorbable indicator. A reference diet (Table 1) was formulated according to Bureau and Cho (1994). Additionally, one treatment included the protease enzyme (inclusion rate of 0.25g enzyme kg diet⁻¹) with the reference diet.

The experimental diets were formulated using 70% of the reference diet with 30% of the experimental ingredient (dry matter basis). The diets were cold extruded, dried in a forced air oven (55°C, 12 h), chopped and screened to obtain the appropriate pellet size. The fish were adapted to the experimental diets for seven days and fecal material collected over a five day period using a settling column which separated the fecal material from the effluent water. Following collection, feces were centrifuged (5000 X g, 15 min), frozen and freeze dried.

Laboratory Analysis

Experimental diets and fecal material were analyzed for moisture (AOAC 1990, method no. 934.01), energy (oxygen bomb calorimetry; Parr Adiabatic Calorimeter, Model 1200), and acid ether extract (AOAC 1995, method no. 954.02). Nitrogen content was determined by combustion method (AOAC 1995). Protein was estimated by multiplying nitrogen content by 6.25. Determination of acid insoluble ash was performed according to the following procedure. Samples (quadruple replicates) were charred (250°C, 18 h), followed by a gradual increase in temperature to 500°C (3 h), and then ashed (48 hours). Four mL of 4N HCl was added and the samples were heated at 120°C for a minimum of one hour. Samples were then centrifuged (3000 x g, 10 min) followed by aspiration of the supernatant. Five mL of water was added, vortexed, centrifuged and aspirated two times. The samples were then dried overnight (80°C) followed by ashing (500°C, 24 h).

Digestibility Calculations

The ADC (%) for the reference and experimental diets were calculated as follows:

$$ADC = 1 - (F/D \times Di/Fi)$$

- Where:
- D = % nutrient in the diet (dry matter (DM) basis)
 - F = % nutrient in the feces (DM basis)
 - Di = % indicator in the diet (DM basis)
 - Fi = % indicator in the feces (DM basis)

The ADC of the test ingredient was calculated as follows (Sugiura et al. 1998):

$$\text{ADCI} = \text{ADCT} + ((1-s) \text{DR}/s \text{DI}) (\text{ADCT} - \text{ADCR})$$

Where: ADCI = Apparent digestibility coefficient of test ingredient

ADCT = Apparent digestibility coefficient of test diet

ADCR = Apparent digestibility coefficient of the reference diet

DR = % nutrient (or kJ/g gross energy) of the reference diet mash
(DM basis)

DI = % nutrient (or kJ/g gross energy) of the test ingredient (DM
basis)

s = Proportion of test ingredient in test diet mash (DM basis)

Analysis of the results used the General Linear Model procedure of SAS (SAS Version 8.0). Mean values were separated using the Student-Newman-Keuls test with the accepted level of significance at $P < 0.05$.

Results:

The ingredient and experimental diet nutrient composition are reported in Table 2 and 3, respectively. The apparent dry matter, crude protein, acid ether extract and energy digestibility of the experimental ingredients are reported in Table 4.

The apparent crude protein digestibility of the dehulled flaxseed was low (63.1%). There was no increase in protein digestibility of dehulled flaxseed with the addition of protease (61.6%; $P > 0.05$).

The lipid digestibility of dehulled flaxseed was very low (46.4%). There was no significant effect ($P > 0.05$) on lipid digestibility of dehulled flaxseed with the addition of the protease enzyme.

The energy digestibility of dehulled flaxseed was very low (49.6%). The addition of the protease to dehulled flaxseed did not improve energy digestibility of this ingredient (47.7%; $P > 0.05$).

The dry matter digestibility of dehulled flaxseed was low (55.4%). The addition of protease to dehulled flaxseed had no impact on dry matter digestibility ($P > 0.05$).

Conclusion:

➤ The results of this experiment indicate that in rainbow trout autoclaved dehulled flaxseed has very poor protein, lipid, energy and overall dry matter digestibility.

➤ Additionally, there is no improvement in nutrient digestibility within rainbow trout with the addition of a protease enzyme to the dehulled flaxseed ingredient.

Table 1. Composition of reference diet utilized in the digestibility study

Ingredient	Inclusion (g kg ⁻¹)
Fishmeal ^a	300
Soybean meal	170
Corn gluten meal	130
Wheat flour	280
Vit./Min. premix ^b	10
Celite ^c	10
Fishoil ^d	100
(Total)	1000

^aSouth American Aquagrade; EWOS Canada Ltd.

^bThe vitamin/mineral premix was a commercial premix (EWOS; closed formulation) formulated to meet the requirements of juvenile rainbow trout.

^cCelite 545, <125µm; Celite Corporation, World Minerals Co., Lompoc, CA, USA

^dMixed variety fish oil; EWOS Canada Ltd.

Table 2. Nutrient analysis of dehulled flaxseed (%)

	Dehulled flaxseed
Dry matter	92.3
Gross energy (kcal kg ⁻¹) ^a	6687.2
Crude protein ^a	24.5
Acid ether extract ^a	32.8

^aDry matter basis

Table 3. Nutrient analysis of the experimental diets (%)

	Diet 1 (Dehulled flaxseed)	Diet 2 (Dehulled flaxseed with protease)	Diet 3 (Reference)	Diet 4 (Reference with protease)
Dry matter	95.7	95.5	93.8	94.9
Gross energy (kcal kg ⁻¹) ^a	5805.7	5797.0	5349.8	5335.4
Crude protein ^a	39.3	39.2	46.1	45.8
Acid ether extract ^a	23.1	23.9	17.1	17.0
Acid insoluble ash ^a	0.90	0.88	1.26	1.27

^aDry matter basis**Table 4. Apparent digestibility coefficients (ADC, %) of dehulled flaxseed and dehulled flaxseed with protease in rainbow trout (n=4)**

	Dehulled flaxseed	Dehulled flaxseed with protease	SEM
Crude protein	63.1 ^a	61.6 ^a	1.54
Acid ether extract	46.4 ^a	46.8 ^a	2.07
Gross energy	49.6 ^a	47.7 ^a	2.09
Dry matter	55.4 ^a	51.8 ^a	2.16

^aMeans in the same row with similar superscripts are similar (P>0.05).

References:

- AOAC (Association of Official Analytical Chemists). 1990.** *Official Methods of Analysis of the Association of Official Analytical Chemists Association of Official Analytical Chemists.* 15th edn. Arlington, VA.
- AOAC (Association of Official Analytical Chemists). 1995.** *Official Methods of Analysis of the Association of Official Analytical Chemists Association of Official Analytical Chemists.* 16th edn. Arlington, VA.
- Bureau, D.P. and Cho, C.Y. 1994.** Ingredient quality: An essential factor in the formulation of cost-effective aquaculture diets. *In* Expanding Agriculture Co-product Uses in Aquaculture Feeds Workshop Proceedings. Des Moines, Iowa, pp. 234-258.
- CCAC (Canadian Council on Animal Care). 1984.** Guide to the Care and Use of Experimental Animals. *In* Canadian Council on Animal Care, Ottawa, ON. Volume 2, pp. 30-45.
- Sugiura, S.D., Dong, F.M., Rathbone, C.K., Hardy, R.W. 1998.** Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. *Aquaculture* 159: 177-202.

Appendix II

Experimental File Number:

PARC 0308

Experimental Title:

Growth performance of rainbow trout (*Oncorhynchus mykiss*) fed CPC canola protein concentrate as a replacement for fish meal.

Experimental Date:

Start date: August 12, 2003

Completion date: November 4, 2003

Researchers:

M. Drew, D. Thiessen

Objectives:

- To evaluate the potential of a mixture of insoluble and soluble canola protein concentrate (CPC) as a replacement for fish meal in a commercial diet for rainbow trout.
- To evaluate the effect of adding a feed stimulant on the intake by rainbow trout of a diet containing 28% CPC and 10% fishmeal.

Material and Methods:

The reference diets consisted of a typical commercial mixture of fishmeal and vegetable sources of protein (40% fishmeal, 17% corn gluten meal, 10% soybean meal and 18% wheat flour). In the test diets, corn gluten meal and soybean meal levels were maintained and CPC was included at 14% (replacing 38% of fishmeal protein), and 28% (replacing 76% of fishmeal protein). At the high level of CPC in the diet, an addition treatment with 1.5% Finnstim was used.

Diet 1 - Fish meal diet (reference diet)

Diet 2 - 14% CPC inclusion

Diet 3 - 28% CPC inclusion

Diet 4 - 28% CPC + Finnstim

The diet formulations are shown in Table 1. The nutrient analysis of experimental diets and the experimental ingredients are shown in Table 2 and 3. Diets were formulated to contain 49% digestible protein (DM basis) and a digestible protein to digestible energy ratio of 22 g MJ⁻¹. Diets were balanced for crude protein, energy and lysine. The diets were cold extruded (3.0 mm die), dried in a forced air oven (55°C, 24 h), chopped and screened to obtain the appropriate size pellet.

Table 1. Reference and Experimental Diets.

Diets	Diet 1 Fish meal reference (g/kg)	Diet 2 14% CPC (g/kg)	Diet 3 28 % CPC (g/kg)	Diet 4 28% CPC + Finnstim (g/kg)
Fishmeal (EWOS mixed)	400	250	100	100
Corn gluten meal	170	170	170	170
Wheat flour	180	189	188	174
CPC	0	137	279	279
Soybean meal	100	100	100	100
Finnstim	0	0	0	15
Fish oil	140	142	149	149
Lysine	0	2	4	3
Choline	5	5	5	5
Vitamin premix	2.5	2.5	2.5	2.5
Mineral premix	2.5	2.5	2.5	2.5
TOTAL as is	1000	1000	1000	1000
DRY g/kg dry diet	917.2	923.9	931.8	933.5
Protein g/kg (as-is)	450.8	453.9	459.4	456.5
Protein g/kg (DM)	491.5	491.4	493.1	488.9
Dig. Prot. g/kg diet (DM)	442.2	442.1	443.9	440.3
Lipid g/kg diet (DM)	200.1	186.0	177.3	176.8
fiber g/kg diet (DM)	18.3	21.2	23.2	22.7
Dig. Energy MJ/kg diet (DM)	19.9	19.8	19.7	19.6
DP/DE g/MJ	22.2	22.4	22.5	22.5
Fish lipid (meal+oil) g/kg (DM)	180.6	167.2	159.0	159.0
fishmeal protein g/kg (DM)	297.4	184.6	73.2	73.0
% of dietary protein	60.5	37.6	14.8	14.9
CPC protein g/kg (DM)	0.0	110.6	223.4	222.9
% of dietary protein	0.0	22.5	45.3	45.6
CGM protein g/kg (DM)	117.0	116.1	115.1	114.9
% of dietary protein	25.9	25.6	25.1	25.2
SBM protein g/kg (DM)	49.8	49.5	49.0	48.9
% of dietary protein	10.1	10.1	9.9	10.0
Wheat protein g/kg (DM)	27.3	28.4	28.0	25.9
% of dietary protein	5.6	5.8	5.7	5.3

Rainbow trout (rainbow/steelhead strain) were acquired from Cangro Processors Ltd. (Lucky Lake, SK, Canada). The fish were maintained in 120 L tanks that were part of a recirculating system using biological filtration. Water temperature was maintained at 14 ± 1 °C. Dissolved oxygen, nitrate, nitrite, ammonia and pH were monitored regularly. Photoperiod was a 14 h light/10 h dark cycle. The fish were maintained in accordance with the guidelines of the Canadian Council on Animal Care (CCAC 1984). A total of 360 rainbow trout weighing 33.3 g

on average were randomly assigned to 24 tanks (15 fish per tank). Six replicates were used for each diet.

The fish were fed by hand to satiation twice daily. The experimental period was 84 days. The total tank weight of the fish was recorded at 28 day intervals for the duration of the experiment. Feed intake was recorded daily. Growth performance was determined by weight gain (g fish^{-1}), total feed intake (g 84 days^{-1}), feed conversion ratio (feed intake wet weight gain $^{-1}$), protein efficiency ratio (wet weight gain protein intake $^{-1}$) and specific growth rate ($[\ln \text{ final weight} - \ln \text{ initial weight}] \text{ time}^{-1} (\text{days}) \times 100$).

Analysis of the results was done using the General Linear Model procedure of SAS (SAS Version 8.2). Differences between treatment means were determined using the Student-Newman-Keuls test at the 0.05 level of significance.

Table 2. Nutrient analysis of dietary ingredients (%)

	CPC	Corn gluten meal	Fish meal	Soybean meal	Wheat flour
Dry matter	94.9	90.8	91.8	89.9	86.5
Gross energy (kcal kg^{-1}) ^a	4991.4	5601.1	4915.8	4788.4	4409.0
Crude protein ^a	76.4	69.5	74.3	50.8	16.1
Ether extract ^a	0.3	2.4	10.1	1.2	0.9
Ash ^a	8.8	1.7	15.6	7.0	2.3

^aDry matter basis

Table 3. Nutrient analysis of the experimental diets (%)

	Diet 1 (ref diet)	Diet 2 (14% CPC)	Diet 3 (28% CPC)	Diet 4 (28% CPC + Finnstim)
Dry matter	95.7	94.7	94.7	94.9
Crude protein ^a	47.4	47.7	47.7	49.9
Ether extract ^a	19.7	18.4	17.4	15.9
Gross energy (kcal kg^{-1}) ^a	5589.5	5653.0	5637.3	5628.8

^aDry matter basis

Results:

Feed Intake. Weekly feed intake is shown in table 4. No difference in week feed intake was found at any time during the trial between fish on the reference diet and on the 14% CPC test diet. Feed intake was significantly lower in fish on the 28% CPC diet over the first 4 weeks of feedings, however, the fish appeared to adapt to the high CPC/low fish meal diet and no differences in feed intake were found among any of the treatment from weeks 5-12 of the trial.

There was no evidence of enhanced feed intake with 1.5% finnstim included in the 28% CPC diet.

Weight Gain. Weight gain at 4, 8 and 12 weeks of feeding is shown in table 5. No statistically significant difference in weight gain was seen between fish on the reference diet and fish on the 14% CPC diet and at 12 weeks of feeding weight gain on the 14% CPC diet was 94% of control weight gain. At 4 weeks of feeding, fish on the 24% CPC diet had significant lower weight gains than fish on the reference diet. No statistically significant differences in weight gain were seen at 8 and 12 weeks of feeding between fish on the reference diet and fish on the 24% CPC diet. Numerically, fish on the 24% CPC diet had weight gains that were 78% of controls at 4 weeks of feeding, 87% of controls at 8 weeks of feeding and 88% of controls at 12 weeks of feeding. This data is consistent with an initial adaptation to the very low fishmeal diet during the first 4 weeks of feeding. Inclusion of an attractant had no effect on weight.

Specific Growth Rates. Specific growth rates at 4, 8 and 12 weeks of feeding are provided in table 5. At 4 weeks of feeding specific growth rates are lower for fish on the 3 experimental diets when compared to controls. However, no statistically significant differences in specific growth rates were seen at 8 and 12 weeks of feeding. This data is consistent with an adaptation period in the first 4 weeks of feeding the high CPC/low fishmeal diets.

Feed Conversion Rates. Table 5 shows feed conversion rates over the 12 week feeding period. Conversion of feed to gain was higher in fish on the reference diets in comparison to the experimental diets at 4, 8 and 12 weeks of feeding. Numerically the differences in feed conversion were largest during the first 4 weeks of feeding. Feed conversion ratio of the fish on the reference diet were 91% of that obtained with fish on the 14% CPC diet and 89% of that obtained with fish on 28% CPC diet. The data suggests an adaptation period in that the numerical differences between feed conversion were higher at 4 weeks of feeding when compared to 8 and 12 weeks.

Protein Efficiency Ratios. Protein efficiency ratios are provided in table 5. Weight gain per weight of protein consumed was higher in the reference diet when compared to the test diets at 4, 8 and 12 weeks of feeding. As observed for feed conversion rate and specific growth rates these differences were higher over the first 4 weeks of feeding and by 12 weeks of feeding the difference in protein efficiency had decreased to 10% for fish on the 14% CPC diet.

Conclusions

1. Replacing 38% and 76% of the fishmeal in commercial type complex diets with canola protein content does not affect feed intake.
2. Incorporation of an attractant in a diet with 28% CPC and 10% fishmeal has no effect on feed intake or any other performance parameter measured in this trial.
3. Incorporation of 14% CPC as a fishmeal replacement in commercial type complex diets has no effect statistically significant effect on weight gain and weight gain at 12 weeks of feeding was 94% of controls.
4. Incorporation of 28% CPC and reducing fishmeal inclusion levels to 10% decreased weight gain during the first 4 weeks but no statistically significant differences were obtained in

weight gain at 8 and 12 weeks of feeding. Numerically weight gain of fish on the 28% CPC diet were 88% of the weight gain of fish on the reference diet.

5. Feed conversion rates and protein efficiency ratios were superior for fish on the reference diet. These differences were highest at 4 weeks of feeding and decreased at 8 and 12 weeks of feeding.

Potential Commercial Use of Canola Protein Concentrates. This trial and previous work with canola protein concentrates demonstrates the potential for use of this feed ingredient as a replacement for fishmeal in commercial diets for carnivorous fish. A conservative initial 10% inclusion rate in a diet containing 17% corn gluten meal and 10% soybean meal would reduce fishmeal levels in the diet by 25%. Substantive differences in performance at this level of fishmeal replacement are unlikely and attractants are not needed to maintain feed intake. The potential for higher levels of inclusion of canola protein concentrate is apparent. A phased approach to increasing the level of canola protein concentrate in the diet may avoid the apparent adaptation period that slows performance during the first 4 weeks of feeding diet containing only 10% fishmeal. In this way it may well be possible to achieve very high levels of fishmeal replacement in the latter growth periods when feed intake is highest.

Table 4. Feed Intake of rainbow trout fed canola protein concentrate for 84 days.

<i>Diet</i>	Feed intake (g fish ⁻¹)											
	<i>Week 1</i>	<i>Week 2</i>	<i>Week 3</i>	<i>Week 4</i>	<i>Week 5</i>	<i>Week 6</i>	<i>Week 7</i>	<i>Week 8</i>	<i>Week 9</i>	<i>Week 10</i>	<i>Week 11</i>	<i>Week 12</i>
Fish meal control	6.3 ^a	14.3 ^a	23.6 ^a	32.3 ^{ab}	42.8 ^a	56.6 ^a	69.9 ^a	84.6 ^a	96.2 ^a	115.3 ^a	132.0 ^a	149.0 ^a
CPC low	6.3 ^a	14.4 ^a	23.2 ^a	33.7 ^a	44.8 ^a	59.0 ^a	73.3 ^a	88.4 ^a	101.0 ^a	118.5 ^a	135.1 ^a	152.6 ^a
CPC high	5.2 ^b	12.2 ^b	20.4 ^b	29.8 ^b	39.8 ^a	53.0 ^a	66.5 ^a	81.1 ^a	94.2 ^a	111.2 ^a	128.0 ^a	145.9 ^a
CPC Finnstim	5.5 ^b	12.7 ^{ab}	21.0 ^{ab}	31.2 ^{ab}	41.6 ^a	55.3 ^a	69.3 ^a	84.3 ^a	97.6 ^a	115.7 ^a	133.2 ^a	150.5 ^a
SEM ¹	0.20	0.53	0.78	0.95	1.27	1.83	2.32	2.85	3.43	3.82	4.39	4.39
<i>Contrasts</i>												
Control vs CPC	<0.05	NS	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS
High vs Low CPC	<0.001	<0.01	<0.05	<0.01	<0.05	<0.05	<0.05	NS	NS	NS	NS	NS
CPC high vs CPC Finnstim	NS ²	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

^{ab}Means in the same row with similar superscripts are similar ($P>0.05$). ¹SEM denotes standard error of the mean. ²denotes non significance ($P>0.05$).

Table 5. The performance of rainbow trout fed canola protein concentrate for 84 days.

<i>Diet</i>	Weight Gain (g fish ⁻¹)			SGR ¹ (%)			FCR ² (g feed g gain ⁻¹)			PER ³ (g gain g protein intake ⁻¹)		
	<i>Week 4</i>	<i>Week 8</i>	<i>Week 12</i>	<i>Week 4</i>	<i>Week 8</i>	<i>Week 12</i>	<i>Week 4</i>	<i>Week 8</i>	<i>Week 12</i>	<i>Week 4</i>	<i>Week 8</i>	<i>Week 12</i>
Fish meal control	37.5 ^a	90.7 ^a	156.2 ^a	2.75 ^a	2.38 ^a	2.10 ^a	0.87 ^b	0.94 ^b	0.96 ^b	2.45 ^a	2.26 ^a	2.21 ^a
CPC low	34.3 ^a	86.2 ^a	146.5 ^a	2.47 ^b	2.24 ^a	1.97 ^a	0.99 ^a	1.03 ^a	1.05 ^a	2.12 ^b	2.04 ^b	2.00 ^b
CPC high	28.8 ^b	78.0 ^a	137.3 ^a	2.21 ^b	2.13 ^a	1.92 ^a	1.04 ^a	1.04 ^a	1.07 ^a	2.02 ^{bc}	2.01 ^b	1.97 ^b
CPC Finnstim	29.6 ^b	80.1 ^a	139.0 ^a	2.21 ^b	2.15 ^a	1.92 ^a	1.08 ^a	1.06 ^a	1.09 ^a	1.89 ^c	1.90 ^c	1.85 ^c
SEM ⁴	1.50	3.59	5.85	0.092	0.064	0.051	0.027	0.016	0.019	0.054	0.033	0.034
<i>Contrasts</i>												
Control vs CPC	<0.001	<0.05	<0.05	<0.001	<0.05	<0.05	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Control vs CPC	<0.001	<0.05	<0.05	<0.001	<0.05	<0.05	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
High vs Low CPC	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CC high vs CPC Finnstim	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.05	<0.05

^{abc}Means in the same row with similar superscripts are similar (P>0.05).

¹SGR = (ln final weight/ ln initial weight)/time (days) x 100. ²FCR = Feed conversion ratio= dry feed intake/wet weight gain.

³PER = wet weight gain/protein intake.

⁴SEM denotes standard error of the mean.

⁵NS denotes non significance (P>0.05).

Appendix III

PARC Experimental Log Numbers:

PARC 0401 and PARC 0402

Experimental Title:

Effect of processing on the digestibility of Saskatchewan feed ingredients to Nile tilapia (*Oreochromis niloticus*).

Researchers:

T.L. Borgeson and M.D. Drew

Objective:

To determine the effect of processing of pea, canola and flax on the digestibility of these ingredients in tilapia diets.

Introduction

Globally, there has been an effort to increase the production of farmed tilapias. This effort has led to the production of over 1.2 million metric tons in 2001. With this development, the need for economically and nutritionally sound diets is becoming more prominent. Currently, feeding accounts for over 50% of the total cost of production in intensive systems with protein being the most costly dietary requirement.

Traditionally, prepared fish diets have focused on using fishmeal as the primary source of protein. Because tilapia are herbivorous, the amount of protein coming from plant materials can be increased while lowering fishmeal concentrations. The goal of this study is to determine the digestibility of prairie feed ingredients in both the processed and unprocessed forms.

Materials and Method:

The ingredients tested in these two trials were: fishmeal, soybean meal, whole pea, pea protein concentrate, canola meal, canola protein concentrate, whole flax, dehulled flax, coextruded flax:pea (Linpro) and coextruded full fat canola:pea (Extrapro).

Fish Management

Nile tilapia were maintained in 120 L tanks that were part of a recirculating system using biological filtration. Water temperature was maintained at $28 \pm 1^\circ \text{C}$. Dissolved oxygen, nitrate, nitrite, ammonia and pH were monitored regularly. Photoperiod was a 14 h light/10 h dark cycle. Forty fish per tank were utilized with five replicates per treatment. The fish were maintained in accordance with the guidelines of the Canadian Council on Animal Care (CCAC 1984).

Digestibility Determination

Apparent digestibility coefficients (ADC, %) were measured using an indirect method with diets containing 1% celite as a nonabsorbable indicator. A reference diet (Table 1) was formulated according to Bureau and Cho (1994). Additionally, one treatment included the protease enzyme (inclusion rate of $0.25 \text{ g enzyme kg diet}^{-1}$) with the reference diet.

The experimental diets were formulated using 70% of the reference diet with 30% of the experimental ingredient (dry matter basis). The diets were cold extruded, dried in a forced air oven (55°C , 12 h), chopped and screened to obtain the appropriate pellet size. The fish were adapted to the experimental diets for seven days and fecal material collected over a five day period using a settling column which separated the fecal material from the effluent water. Following collection, feces were centrifuged ($5000 \times g$, 15 min), frozen and freeze dried.

Laboratory Analysis

Experimental diets and fecal material were analyzed for moisture (AOAC 1990, method no. 934.01), energy (oxygen bomb calorimetry; Parr Adiabatic Calorimeter, Model 1200), and acid ether extract (AOAC 1995, method no. 954.02). Nitrogen content was determined by combustion method (AOAC 1995). Protein was estimated by multiplying nitrogen content by 6.25. Determination of acid insoluble ash was performed according to the following procedure. Samples (quadruplicate replicates) were charred (250°C , 18 h), followed by a gradual increase in temperature to 500°C (3 h), and then ashed (48 hours). Four mL of 4N HCl was added and the samples were heated at 120°C

for a minimum of one hour. Samples were then centrifuged (3000 x g, 10 min) followed by aspiration of the supernatant. Five mL of water was added, vortexed, centrifuged and aspirated two times. The samples were then dried overnight (80°C) followed by ashing (500°C, 24 h).

Digestibility Calculations

The ADC (%) for the reference and experimental diets were calculated as follows:

$$ADC = 1 - (F/D \times Di/Fi)$$

Where: D = % nutrient in the diet (dry matter (DM) basis)

F = % nutrient in the feces (DM basis)

Di = % indicator in the diet (DM basis)

Fi = % indicator in the feces (DM basis)

The ADC of the test ingredient was calculated as follows (Sugiura et al. 1998):

$$ADCI = ADCT + ((1-s) DR/s DI) (ADCT - ADCR)$$

Where: ADCI = Apparent digestibility coefficient of test ingredient

ADCT = Apparent digestibility coefficient of test diet

ADCR = Apparent digestibility coefficient of the reference diet

DR = % nutrient (or kJ/g gross energy) of the reference diet mash
(DM basis)

DI = % nutrient (or kJ/g gross energy) of the test ingredient (DM
basis)

s = Proportion of test ingredient in test diet mash (DM basis)

Analysis of the results used the General Linear Model procedure of SAS (SAS Version 8.0). Mean values were separated using the Student-Newman-Keuls test with the accepted level of significance at $P < 0.05$.

Results:

The experimental diet nutrient composition is reported in Table 2 and the apparent dry matter, crude protein, acid ether extract and energy digestibility of the experimental ingredients are reported in Table 3.

- Dehulled flax and whole flax had significantly lower ADCs for protein compared to other ingredients
- Pea and canola protein concentrates had a significantly improved ADCs for energy and dry matter compared with unprocessed pea and canola. The protein digestibility was not significantly improved by processing.
- Extrapro and Linpro had significantly lower ADCs for energy and dry matter than canola or pea protein concentrates.

Conclusion:

- Processing methods such as dehulling, aqueous extraction and extrusion can significantly improve ADCs for energy and dry matter but not crude protein in pea, canola and flax by tilapia

Table 1. Composition of reference diet utilized in the digestibility study

Ingredient	Inclusion (g kg ⁻¹)
Fishmeal ^a	300
Soybean meal	170
Corn gluten meal	130
Wheat flour	280
Vit./Min. premix ^b	10
Celite ^c	10
Fishoil ^d	100
(Total)	1000

^aSouth American Aquagrade; EWOS Canada Ltd.

^bThe vitamin/mineral premix was a commercial premix (EWOS; closed formulation) formulated to meet the requirements of juvenile rainbow trout.

^cCelite 545, <125µm; Celite Corporation, World Minerals Co., Lompoc, CA, USA

^dMixed variety fish oil; EWOS Canada Ltd.

Table 2. Nutrient analysis of the experimental diets.

Diet	Dry Matter ^a	Crude Protein ^a	Gross Energy ^a	Acid Ether Extract ^a	Ash ^a	Acid Insoluble Ash ^a
Fishmeal	96.11	51.80	5205.47	15.28	11.27	.96
Soybean Meal	96.12	45.97	5108.90	12.30	7.97	1.06
Whole Pea	95.64	37.21	4948.09	8.86	6.77	.98
Pea Protein Concentrate	95.63	55.13	5363.11	14.50	7.53	.93
Canola Meal	94.75	44.37	5236.74	14.59	8.23	1.08
Canola Protein Concentrate	96.50	51.91	5193.69	13.00	9.18	1.04
Extrapro	96.39	37.95	5330.51	16.95	7.00	1.07
Whole Flax	95.69	36.67	5638.95	15.50	6.85	1.33
Dehulled Flax	97.20	36.76	5805.96	27.34	6.68	.98
Linpro	96.35	37.77	5339.88	19.16	6.92	1.48
Control	96.89	43.32	5278.43	17.82	8.17	1.27

^aDry Matter basis

Table 4. Apparent digestibility coefficients (ADC, %) of ingredients fed to Nile tilapia.

Ingredient	Crude Protein	Gross Energy	Dry Matter
Fishmeal	.93 ^a	.92 ^a	.85 ^{ab}
Soybean Meal	.91 ^a	.82 ^{ab}	.74 ^{abcd}
Whole Pea	.86 ^a	.58 ^{cd}	.59 ^{cde}
Pea Protein Concentrate	.95 ^a	.95 ^a	.93 ^a
Canola Meal	.82 ^a	.68 ^{bc}	.54 ^{de}
Canola Protein Concentrate	.86 ^a	.84 ^{ab}	.78 ^{abc}
Extrapro	.61 ^b	.53 ^{cd}	.41 ^e
Whole Flax	-.38 ^d	-.27 ^e	-.45 ^f
Dehulled Flax	.46 ^c	.48 ^d	.41 ^e
Linpro	.76 ^{ab}	.84 ^a	.69 ^{bcd}

^{a-f} Different letters denote significant differences within a column (P < 0.05)

Means separation by Student-Newman-Keuls test

References:

- AOAC (Association of Official Analytical Chemists). 1990.** *Official Methods of Analysis of the Association of Official Analytical Chemists Association of Official Analytical Chemists.* 15th edn. Arlington, VA.
- AOAC (Association of Official Analytical Chemists). 1995.** *Official Methods of Analysis of the Association of Official Analytical Chemists Association of Official Analytical Chemists.* 16th edn. Arlington, VA.
- Bureau, D.P. and Cho, C.Y. 1994.** Ingredient quality: An essential factor in the formulation of cost-effective aquaculture diets. *In* Expanding Agriculture Co-product Uses in Aquaculture Feeds Workshop Proceedings. Des Moines, Iowa, pp. 234-258.
- CCAC (Canadian Council on Animal Care). 1984.** Guide to the Care and Use of Experimental Animals. *In* Canadian Council on Animal Care, Ottawa, ON. Volume 2, pp. 30-45.
- Sugiura, S.D., Dong, F.M., Rathbone, C.K., Hardy, R.W. 1998.** Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. *Aquaculture* 159: 177-202.

Appendix IV

Experimental File Number:

PARC 0403

Experimental Title:

Growth performance of Nile tilapia (*Oreochromis nilotica*) fed simple or complex mixtures of vegetable protein replacing fish meal I.

Researchers:

T. Borgeson, M. Drew, L. White

Objective:

To determine the effect of replacing fish meal with a simple (a mixture of soybean meal and corn gluten meal) or complex (a mixture of canola protein concentrate, pea protein concentrate, dehulled flax, soybean meal and corn gluten meal) mixture of plant proteins in diets fed to Nile Tilapia.

Materials and Methods

Fifty six tanks of tilapia were randomly assigned to one of 7 different diets shown in Table 1. All diets were balanced for digestible crude protein (36%) and digestible energy (1.64 MJ/kg). The proximate and amino acid analyses of the diets are shown in Tables 2 and 3 respectively. The control diet contained fish meal as the primary source of protein. The fish meal in the control diet was replaced with a mixture of soybean meal and corn gluten meal (Simple), canola protein concentrate (CPC) or a mixture of soybean meal, corn gluten meal, pea protein concentrate, dehulled flax and canola protein concentrate (Complex). The vegetable protein mixtures were used to replace 0, 33, 66 or 100% of the fish meal protein in a 2 x 4 factorial design. The diets were mixed using a Hobart mixer and then extruded at the Food Processing Center in Edmonton with Alberta Agriculture, Food and Rural Revitalization using a twin screw extruder. The extruded feed was dried in a continuous fluid bed dryer and fish oil was added after the diets were dry using a vacuum sealed rotating drum.

Nile tilapia were acquired from Greenview aquafarms (Calgary AB). The fish were maintained in 120 L tanks that were part of a recirculating system using biological filtration. Water temperature was maintained at 28 ± 1 °C. Dissolved oxygen, nitrate, nitrite, ammonia and pH were monitored regularly. Photoperiod was a 14 h light/10 h dark cycle. The fish were maintained in accordance with the guidelines of the Canadian Council on Animal Care (CCAC 1984). A total of 560 tilapia weighing 333.5 g on average were randomly assigned to 60 tanks (10 fish per tank). Six replicates were used for each diet.

The fish were fed by hand to satiation twice daily. The experimental period was 63 days. The total tank weight of the fish was recorded at 21 day intervals for the duration of the experiment. Feed intake was recorded daily. Growth performance was determined by

weight gain (g fish^{-1}), average daily feed intake (g/fish/day), feed conversion ratio (feed intake wet weight gain $^{-1}$), protein efficiency ratio (wet weight gain protein intake $^{-1}$) and specific growth rate ($[\ln \text{ final weight} - \ln \text{ initial weight}] \text{ time}^{-1} (\text{days}) \times 100$).

Analysis of the results was done using the General Linear Model procedure of SPSS (Release 12.0). Differences between treatment means were determined using the Student-Newman-Keuls test at the 0.05 level of significance.

Summary of Results

Average Daily Gain (Table 4; Figure 1)

- interaction between fishmeal % and complexity of the diet was not significant $P > 0.05$
- days 0-56 showed a significant difference between 0% fishmeal and 33%, 66%, 100% fishmeal
- complex diets for all days, numerically had higher average daily gains compared with simple diets however, there was no significant difference ($P > 0.05$) between simple and complex
- 100% fishmeal numerically had the highest average daily gain (day 0-56)

Specific Growth Rate (Table 5; Figure 2)

- significant interaction between fishmeal % and complexity of the diet $P < 0.05$
- based on complexity, there was no significant difference between simple and complex ($P > 0.05$)
- 0% Simple had a significant difference in specific growth rate compared to all diets for days 0-56

Average Daily Feed Intake (Table 6; Figure 3)

- significant interaction between fishmeal % and complexity of the diet $P < 0.05$
- 0% fishmeal was significantly different from 33%, 66% and 100% fishmeal
- a significance was seen between complex and simple diets ($P < 0.05$)
- for the entire 56 days, average daily feed intake was equal for all diets with the exception of the 0% Simple diet which was significantly lower

Feed:Gain (Table 7; Figure 4)

- interaction between fishmeal % and complexity of the diet was not significant $P > 0.05$
- feed:gain values were not significantly different throughout the trial until day 42-56 (0% fishmeal significantly different)
- for days 0-56 0% and 33% fishmeal were significantly different from 66% and 100% fishmeal
- based on complexity, simple diets numerically had poorer feed:gain ratio compared to the complex diets for all days with the exception of 0-14 days however, no significant difference was detected ($P > 0.05$)
- analysis of the means by diet did not include the 0% Simple diet because there was not enough data points (fish lost weight resulting in negative feed:gain ratios)

- 33% Simple and 0% Complex were significantly different from 33% Complex, 66% Simple and Complex, and 100%

Protein Efficiency Ratio (Table 8; Figure 5)

- significant interaction between fishmeal % and complexity of the diet $P < 0.05$
- significant difference was found between complex diets and simple diets ($P < 0.05$)
- a significant difference was found with 0% Simple diet on day 14-28, 28-42 and 42-56
- day 0-56 showed 0% Simple with the lowest protein efficiency ratio and 33% Simple with the second lowest while all other diets were considered similar
- based on diet, 100% had the highest protein efficiency ratio, 66% Simple and Complex, 33% Simple and Complex and 0% Complex with the second highest and 0% Simple with the lowest

Conclusions

- overall complex diets had higher average weights, average daily gains, specific growth rates, protein efficiency ratios and lower feed:gain ratios compared to simple diets
- numerically, higher weights are obtained through higher levels of fishmeal but 0% fishmeal is statistically similar to other levels of fishmeal if it is not a simple diet

Table 1. Diet composition used in the experiment.

	<u>Simple</u>				<u>Complex</u>		
	100%FM	66.6%FM	33.3%FM	0%FM	66.6%FM	33.3%FM	0%FM
Fishmeal	14.61	9.74	4.87	0.00	9.74	4.87	0.00
Wheat	6.94	6.22	5.51	4.79	5.93	5.42	4.65
Corn Gluten Meal	0.00	2.84	5.67	8.51	1.67	2.50	3.00
Soybean Meal	0.00	3.00	6.00	9.00	1.00	2.50	4.31
Canola Protein Conc.	0.00	0.00	0.00	0.00	1.00	3.00	6.18
Dehulled Flax	0.00	0.00	0.00	0.00	1.50	1.50	1.50
Pea Protein Conc.	0.00	0.00	0.00	0.00	1.50	2.81	3.00
Choline	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Vit/Min	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Fish Oil	3.08	2.83	2.58	2.33	2.29	2.02	1.99

Table 2. Diet nalysis

Diet	Moisture	Crude Protein^a (%)	Ether Extract^a (%)	Gross Energy^a (cal/g)	Ash^a (%)
100 %	11.03	44.79	13.66	5364.22	10.86
66.6 % Simple	8.77	44.40	18.47	5390.63	8.71
33.3% Simple	6.52	41.44	17.12	5416.25	7.37
0% Simple	13.32	40.37	14.02	5369.68	4.45
66.6% Complex	4.93	45.69	19.65	5483.12	8.80
33.3% Complex	14.05	44.54	17.23	5442.15	6.39
0% Complex	10.20	42.50	15.16	5408.82	6.01

^aDry matter basis

Table 3. Analysis of crude protein and amino acids in experimental diets.

	100%	66.6 % Simple	66.6% Complex	<u>Diet</u> 33.3% Simple	33.3% Complex	0 % Simple	0% Complex
Crude Protein^a	44.79	44.40	45.69	41.44	44.54	40.37	42.50
Amino Acid^a							
<i>Methionine</i>	1.09	0.99	0.99	0.83	0.87	0.74	0.74
<i>Cystine</i>	0.43	0.52	0.54	0.57	0.63	0.62	0.73
<i>Methionine + Cystine</i>	1.52	1.51	1.53	1.40	1.50	1.36	1.47
<i>Lysine</i>	2.73	2.38	2.50	1.86	2.32	1.57	2.01
<i>Arginine</i>	1.70	1.65	1.74	1.50	1.65	1.47	1.61
<i>Threonine</i>	2.71	2.57	2.79	2.22	2.87	2.10	2.73
<i>Isoleucine</i>	1.66	1.74	1.70	1.71	1.79	1.75	1.85
<i>Leucine</i>	2.88	3.57	3.40	4.09	3.69	4.65	3.78
<i>Valine</i>	2.03	2.07	2.04	1.97	2.13	1.98	2.18
<i>Histidine</i>	1.07	1.08	1.10	0.97	1.11	0.95	1.05
<i>Phenylalanine</i>	1.63	1.87	1.86	1.99	1.96	2.16	2.06
<i>Glycine</i>	3.73	3.01	3.13	2.13	2.55	1.61	1.92
<i>Serine</i>	1.86	1.95	2.05	1.92	2.00	2.04	1.98
<i>Alanine</i>	2.73	2.75	2.68	2.61	2.46	2.67	2.20
<i>Aspartic Acid</i>	3.61	3.64	3.76	3.40	3.75	3.43	3.67
<i>Glutamic Acid</i>	6.35	7.16	7.27	7.61	7.84	8.26	8.22

^a % as is

Table 4. Average Daily Gains of fish during the experiment.

% Protein from Fishmeal	<u>Days</u>				
	0-14	14-28	28-42	42-56	0-56
0%	0.76a	1.20a	0.75a	0.65a	0.78a
33%	1.73b	2.08b	1.91b	2.11b	1.67b
66%	2.27b	3.20b	2.23b	2.20b	2.44b
100%	2.97b	2.63b	2.43b	2.21b	2.67b
SE	0.22	0.21	0.17	0.14	0.16
Complexity					
Simple	1.59	1.99	1.50	1.54	1.63
Complex	2.20	2.63	2.18	1.96	2.09
SE	0.22	0.21	0.17	0.14	0.16
FM* Complexity					
Interaction (P Value)	0.09	0.00	0.33	0.30	0.00

Figure 1. Average Daily Gains of fish during the experiment.

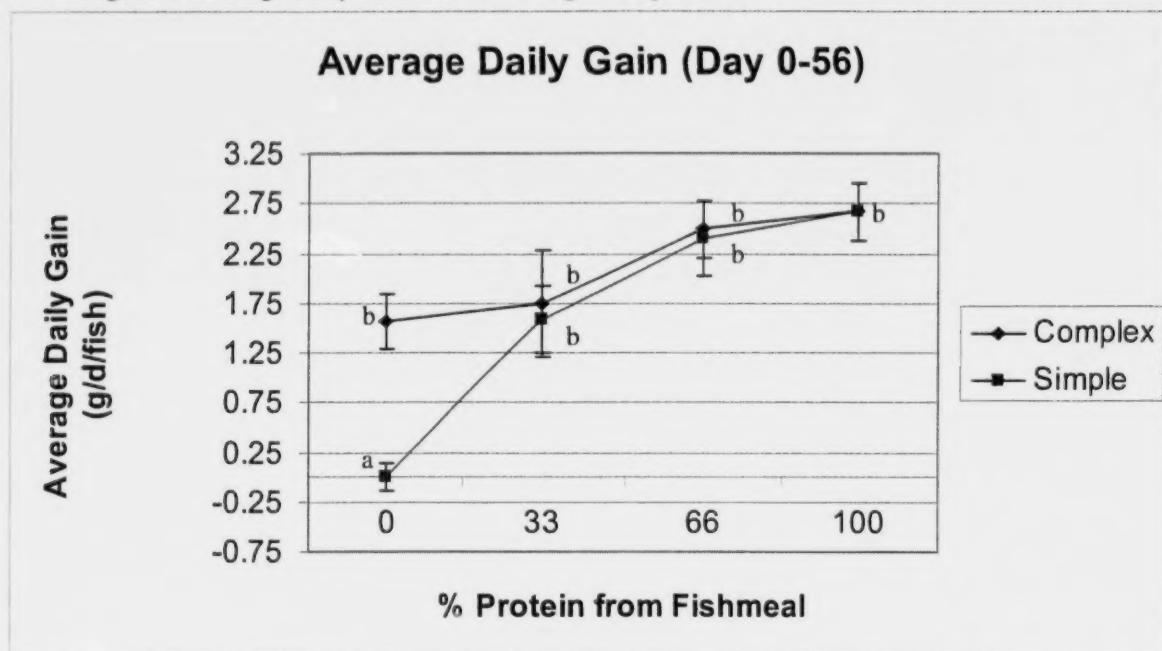


Table 5. Specific Growth Rate of fish during the experiment

% Protein from Fishmeal	<u>Days</u>				
	0-14	14-28	28-42	42-56	0-56
0%	0.47a	0.45a	0.25a	0.31a	0.39a
33%	0.77ab	0.73ab	0.67b	0.65b	0.73b
66%	1.07ab	1.24c	0.72b	0.52ab	0.92b
100%	1.29b	0.94bc	0.75b	0.65b	0.91b
SE	0.10	0.08	0.05	0.04	0.05
Complexity					
Simple	0.75a	0.75a	0.50a	0.46a	0.65a
Complex	1.03b	0.90b	0.68b	0.60b	0.81b
SE	0.10	0.08	0.05	0.04	0.05
% Fishmeal *					
Complexity (P Value)	0.18	0.01	0.34	0.03	0.00

Figure 2. Specific Growth Rate of fish during the experiment

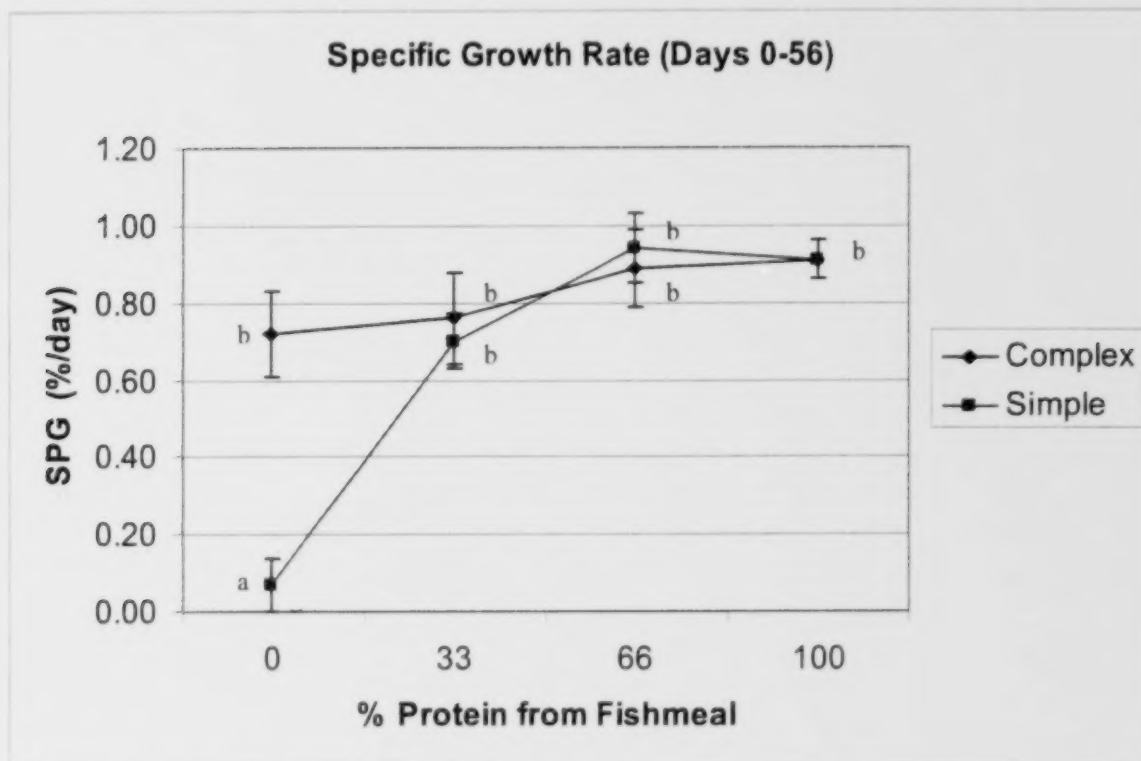


Table 6. Feed:Gain ratios of fish during the experiment.

% Protein from Fishmeal	<u>Days</u>				
	0-14	14-28	28-42	42-56	0-56
0%	1.63a	2.00a	3.68a	3.61b	3.27b
33%	1.84a	2.29a	1.83a	1.87a	1.93b
66%	1.00a	1.24a	1.83a	1.69a	1.34a
100%	0.86a	1.81a	1.89a	1.75a	1.17a
SE	0.17	0.23	0.25	0.24	0.18
Complexity					
Simple	1.19	2.13	2.67	2.46	2.22
Complex	1.42	1.51	1.84	1.98	1.54
SE	0.17	0.23	0.25	0.24	0.18
% Fishmeal *					
Complexity (P Value)	0.94	0.09	0.70	0.75	0.09

Figure 3. Feed:Gain ratios of fish during the experiment.

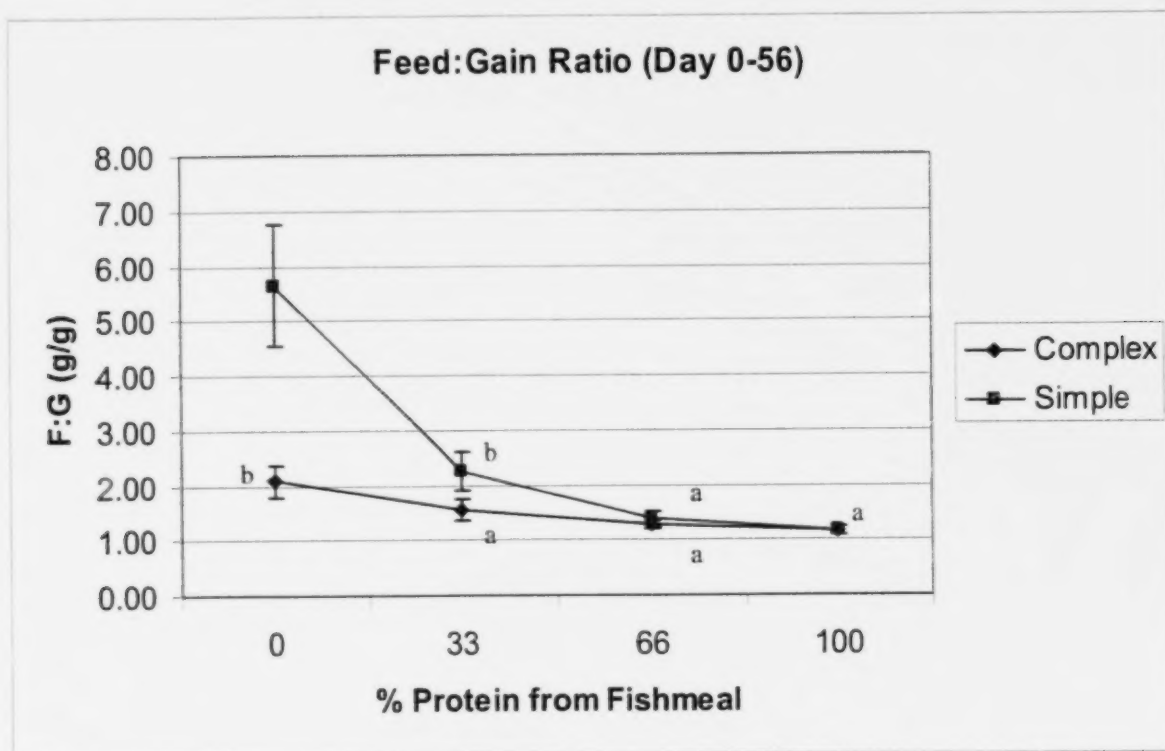


Table 7. Average Daily Feed Intake of fish during the experiment.

% Protein from Fishmeal	<u>Days</u>				
	0-14	14-28	28-42	42-56	0-56
0%	1.54a	2.12a	2.34a	2.69a	2.14a
33%	2.19b	3.14b	3.20b	3.27b	2.85b
66%	2.15b	3.38b	3.31b	3.74b	3.04b
100%	2.34b	3.36b	3.54b	3.40b	3.06b
SE	0.10	0.12	0.13	0.13	0.11
Complexity					
Simple	1.91a	2.76a	2.90a	3.31a	2.59a
Complex	2.18b	3.21b	3.27b	3.22b	2.94b
SE	0.10	0.12	0.13	0.13	0.11
% Fishmeal *					
Complexity (P Value)	0.02	0.00	0.05	0.20	0.02

Figure 4. Average Daily Feed Intake of fish during the experiment.

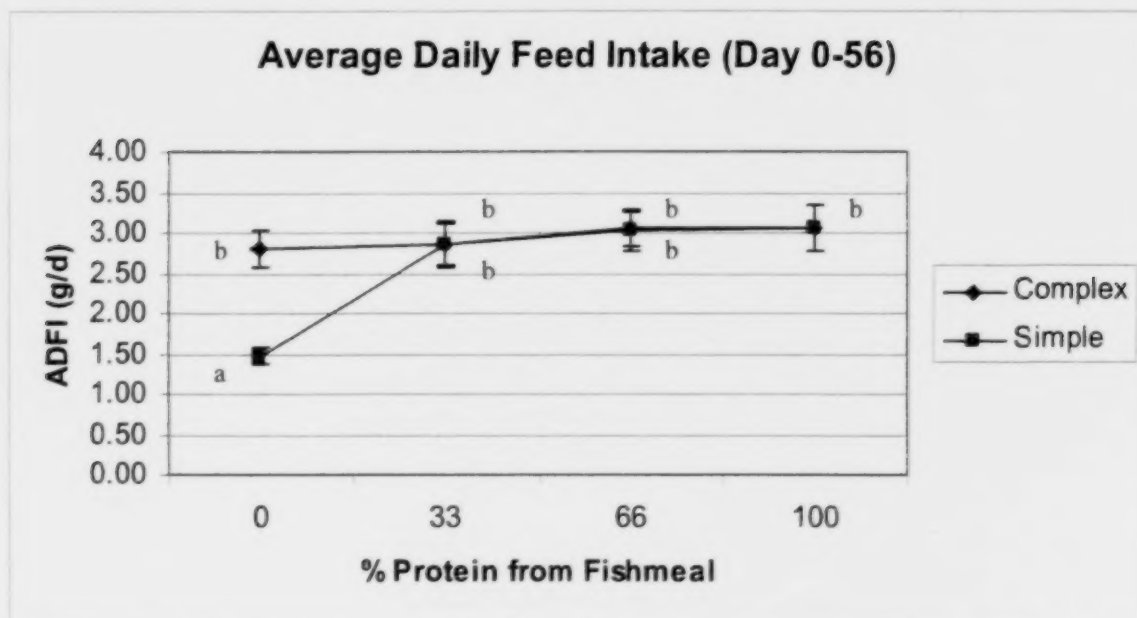
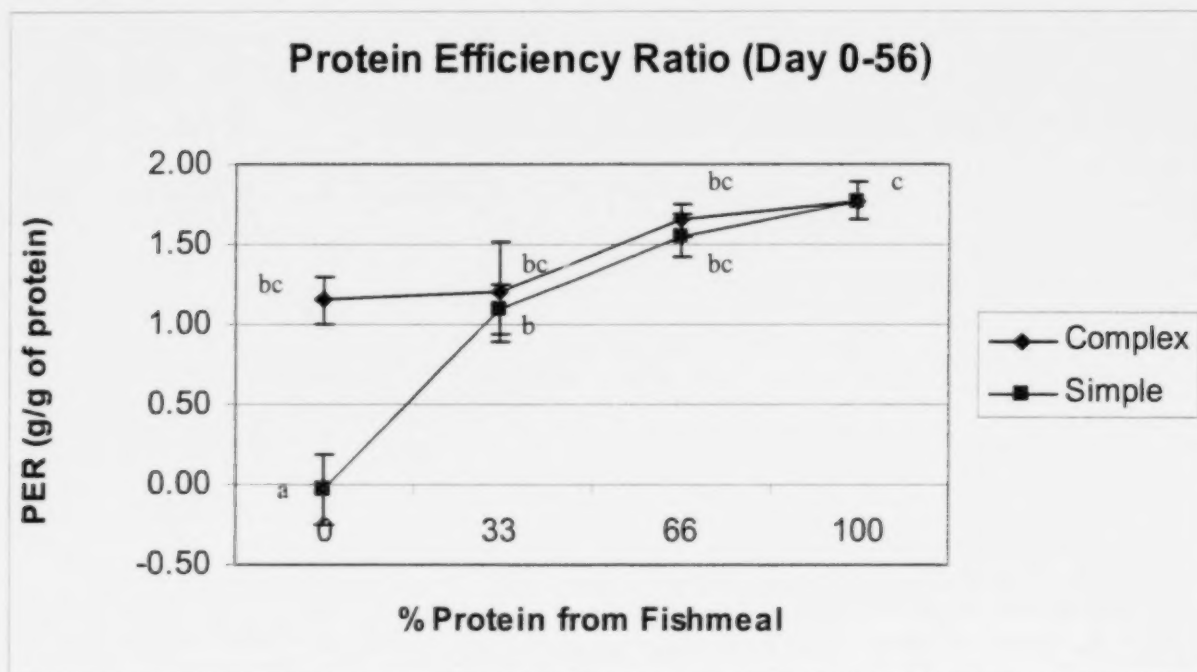


Table 8. Protein Efficiency Ratio of fish during the experiment.

% Protein from Fishmeal	<u>Days</u>				
	0-14	14-28	28-42	42-56	0-56
0%	0.42a	0.79a	0.46a	0.5a	0.56a
33%	1.49a	1.36ab	0.98b	1.32b	1.14b
66%	2.10a	1.88b	1.30b	1.31b	1.59c
100%	2.66a	1.56ab	1.29b	1.37b	1.77c
SE	0.30	0.14	0.09	0.08	0.09
Complexity					
Simple	1.23a	1.18a	0.87a	0.96a	1.07a
Complex	2.04b	1.66b	1.12b	1.25b	1.42b
SE	0.30	0.14	0.09	0.08	0.09
% Fishmeal *					
Complexity (P Value)	0.33	0.01	0.26	0.35	0.00

Table 5. Protein Efficiency Ratio of fish during the experiment.



Appendix V

Experimental File Number:

PARC 0404

Experimental Title:

Growth performance of Nile tilapia (*Oreochromis nilotica*) fed simple or complex mixtures of vegetable protein replacing fish meal.

Researchers:

M. Drew, L. White

Objectives:

- To determine the effect of replacing fish meal with a simple (canola protein concentrate or a mixture of soybean meal and corn gluten meal) or complex (a mixture of canola protein concentrate, soybean meal and corn gluten meal) mixture of plant proteins in diets fed to Nile Tilapia.

Material and Methods:

Sixty tanks of tilapia were randomly assigned to one of 10 different diets shown in Table 1. All diets were balanced for digestible crude protein (36%) and digestible energy (1.64 MJ/kg). The control diet contained fish meal as the primary source of protein. The fish meal in the control diet was replaced with a mixture of soybean meal and corn gluten meal (SBM/CGM), canola protein concentrate (CPC) or a mixture of soybean meal, corn gluten meal and canola protein concentrate (SBM/CGM/CPC). The vegetable protein mixtures were used with 20, 10 or 0% fish meal in a 3 x 4 factorial design. The diets were cold extruded (3.0 mm die), dried in a forced air oven (55°C, 24 h), chopped and screened to obtain the appropriate size pellet.

Nile tilapia were acquired from Greenview aquafarms (Calgary AB). The fish were maintained in 120 L tanks that were part of a recirculating system using biological filtration. Water temperature was maintained at 28 ± 1 °C. Dissolved oxygen, nitrate, nitrite, ammonia and pH were monitored regularly. Photoperiod was a 14 h light/10 h dark cycle. The fish were maintained in accordance with the guidelines of the Canadian Council on Animal Care (CCAC 1984). A total of 600 tilapia weighing xxx g on average were randomly assigned to 56 tanks (10 fish per tank). Eight replicates were used for each diet.

The fish were fed by hand to satiation twice daily. The experimental period was 56 days. The total tank weight of the fish was recorded at 14 day intervals for the duration of the experiment. Feed intake was recorded daily. Growth performance was determined by weight gain (g fish^{-1}), average daily feed intake (g/fish/day), feed conversion ratio (feed intake wet weight gain^{-1}), protein efficiency ratio (wet weight gain protein intake $^{-1}$) and specific growth rate ($[\ln \text{ final weight} - \ln \text{ initial weight}] \text{ time}^{-1} (\text{days}) \times 100$).

Analysis of the results was done using the General Linear Model procedure of SPSS (Release 12.0). Differences between treatment means were determined using the Student-Newman-Keuls test at the 0.05 level of significance.

Results

- The main effects of Fish meal and Diet Complexity interacted significantly ($P = 0.023$) and so tables for the main effects as well as figures for individual dietary treatments are shown.
- Fish on the 20% fish meal diets were the heaviest at the end of the experiment and were significantly heavier than fish on the 0% fish meal diets ($P < 0.05$). Fish fed the 10 and 46% fishmeal diets were not significantly different in weight than the other two groups on day 63 of the experiment (Table 2). The average daily gains and specific growth rates of the fish fed the 20% fish meal diets were significantly higher than those fed the 10 or 0% fish meal diets ($P < 0.05$). The SGRs of the 46% fish meal controls were not significantly different from fish the 20% fish meal.
- The fish fed the SBM/CGM/CPC diets had ADGs and SGRs that were not significantly than those fed diets based on SBM/CGM or CPC alone ($P < 0.05$) and the fish fed the SBM/CGM/CPC diets tended to be heavier than the fish fed the SBM/CGM or CPC diets ($P < 0.1$).
- Average daily feed intakes and feed:gain ratios were significantly reduced in the fish fed the 0% fish meal diets compared to the 20% fish meal diets (Table 3). There were no significant differences in ADFI, F:G or PER values of fish fed the 10, 20 or 46% fish meal diets ($P > 0.05$).
- There were no significant differences in ADFI F:G or PER ratios between the groups fed different plant protein sources. However F:G ratios tended to be better in the fish fed the SBM/CGM/CPC diets compared to the SBM/CGM or CPC diets (Table 3). PER values were not significantly affected by the main effects of fish meal or diet complexity.
- Figure 1 shows SGR of fish fed the individual diets used in the trial. The fish fed the 10% fishmeal diet based on SBM/CGM/CPC had SGRs that were significantly higher than those fed the 10% fish meal diets based on CPC or SBM/CGM ($P < 0.05$) and not significantly different from those fed the 46% fish meal control diet.
- Figure 2 shows the average daily feed intake of fish fed the individual diets used in this trial. There were no significant treatment differences, however, there was a trend to higher feed consumption in the fish fed diets based on SBM/CGM/CPC compared to the other vegetable sources.
- Figure 3 shows the feed:gain ratios of fish fed the individual diets used in this trial. There were no significant treatment differences, however, the feed:gain ratios of the fish fed diets based on SBM/CGM/CPC did not increase with decreasing levels of fish meal in the diet.
- Figure 4 shows the protein efficiency ratios of fish fed the individual diets used in this trial. There were no significant treatment differences, however, the PER values of the fish fed diets based on SBM/CGM/CPC tended to be higher than fish fed diets based on the other vegetable proteins.

Conclusions

Based on the results above:

- The formulation of diets fed to tilapia using a complex mixture of vegetable proteins including soybean meal, corn gluten meal and canola protein concentrate allows a greater percentage of fish meal to be replaced than diets using simpler mixtures of plant proteins without reducing fish growth or feed efficiency.

Table 1. Diets used in the experiment.

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal	46.07	20.00	20.00	20.00	10.00
SBM	~	10.10	16.59	~	15.00
CGM	~	9.80	15.00	~	15.00
CPC	~	10.00	~	27.23	11.64
Wheat	50.39	44.20	41.84	47.93	41.42
Fish oil	1.00	2.90	3.57	1.84	3.93
Canola oil	1.54	2.00	2.00	2.00	2.00
Vitamin/mineral	1.00	1.00	1.00	1.00	1.00
Lysine	~	~	~	~	~
Calculated Analysis:					
Crude protein (%)	41.9	40.7	40.2	41.5	40.1
Digestible protein (%)	36.0	36.0	36.0	36.0	36.0
Digestible energy (MJ)	1.64	1.64	1.64	1.64	1.64
DP:DE (g/MJ)	22.0	22.0	22.0	22.0	22.0
Lysine (%)	3.02	2.26	2.15	2.48	1.94
Lipid (%)	8.41	8.51	9.41	7.05	8.72
Linoleic acid (%)	0.84	1.02	1.12	0.86	1.08

Ingredient	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10
Fish meal	10.00	10.00	~	~	~
SBM	23.94	~	15.00	27.29	~
CGM	20.00	~	15.00	28.29	~
CPC	~	37.68	22.13	~	48.13
Wheat	38.26	46.98	40.33	35.00	46.06
Fish oil	4.81	2.34	4.47	5.98	2.84
Canola oil	2.00	2.00	2.00	2.00	2.00
Vitamin/mineral	1.00	1.00	1.00	1.00	1.00
Lysine	~	~	0.07	0.44	~
Calculated Analysis:					
Crude protein (%)	39.5	41.3	40.0	39.3	41.2
Digestible protein (%)	36.0	36.0	36.0	36.0	36.0
Digestible energy (MJ)	1.64	1.64	1.64	1.64	1.64
DP:DE (g/MJ)	22.0	22.0	22.0	22.0	22.0
Lysine (%)	1.83	2.28	1.80	1.80	2.08
Lipid (%)	9.86	6.55	8.24	10.29	6.02
Linoleic acid (%)	1.18	0.83	1.05	1.26	0.80

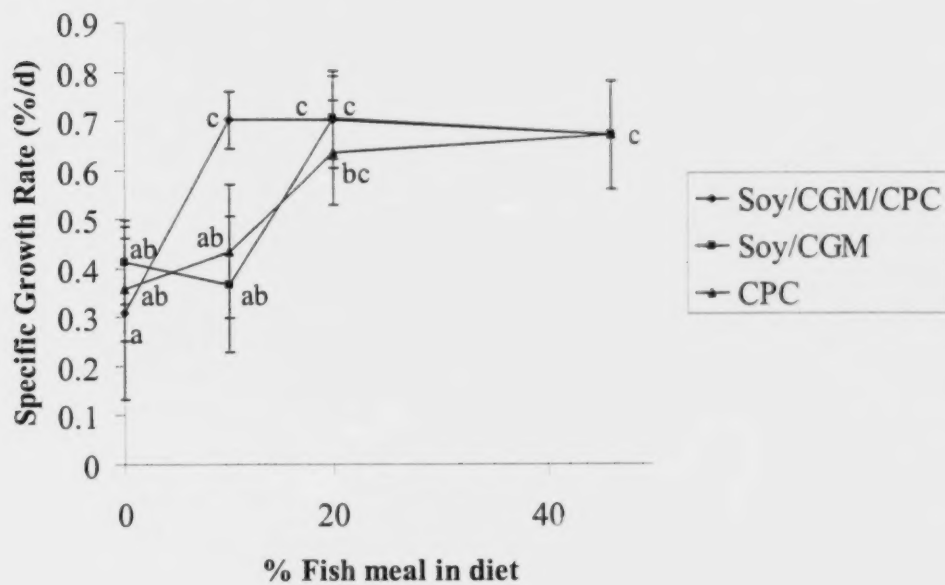
Table 2. Growth performance of tilapia fed diets with simple or complex mixtures of vegetable proteins replacing fish meal.

Fish meal	Fish weight on day				Average Daily Gain (g/d)	Specific Growth Rate (%/d)
	0	21	42	63		
46	326.5	380.9	441.7	503.7ab	2.81ab	0.671ab
20	347.1	420.4	477.3	546.2b	3.02b	0.682b
10	318.6	355.3	409.6	462.6ab	2.41ab	0.501ab
0	336.8	359.9	397.4	445.5a	1.70a	0.360a
Diet Complexity						
SBM/CGM/CPC	342.5	400.2	452.7	518.2	2.96b	0.596b
SBM/CGM	318.6	361.1	410.0	449.3	2.13a	0.495a
CPC	336.1	367.8	417.8	482.0	2.10a	0.475a
SE	10.7	14.9	16.3	18.9	0.19	0.039

Table 3. Feed intake, feed efficiency and protein efficiency ratios of tilapia fed diets with simple or complex mixtures of vegetable proteins replacing fish meal.

Fish meal	Average Daily Feed Intake	Feed:Gain	PER
46	4.02ab	1.63b	0.92
20	4.71b	1.95b	1.01
10	4.04ab	2.42ab	0.86
0	3.44a	3.03a	0.79
Diet Complexity			
SBM/CGM/CPC	4.35	1.70	0.90
SBM/CGM	3.83	2.71	0.86
CPC	3.90	2.86	0.85
SE	0.17	0.63	0.05

Figure 1. Specific growth rate of tilapia fed diets with simple or complex mixtures of vegetable proteins replacing fish meal.



^{abc}Data points with different superscripts are significantly different ($P < 0.05$).

Figure 2. Average daily feed intake of tilapia fed diets with simple or complex mixtures of vegetable proteins replacing fish meal.

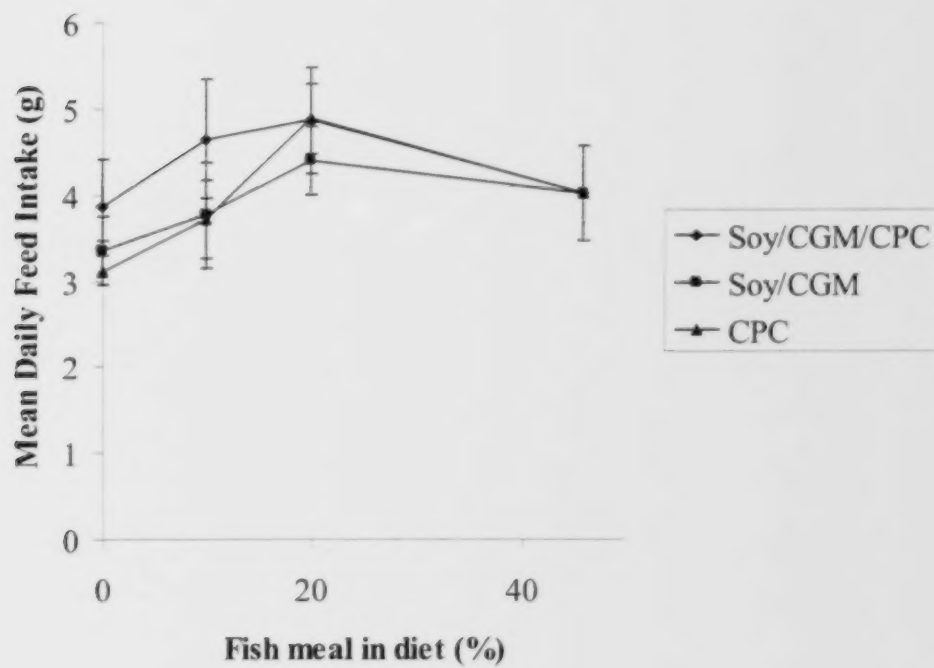


Figure 3. Feed:gain ratios of tilapia fed diets with simple or complex mixtures of vegetable proteins replacing fish meal.

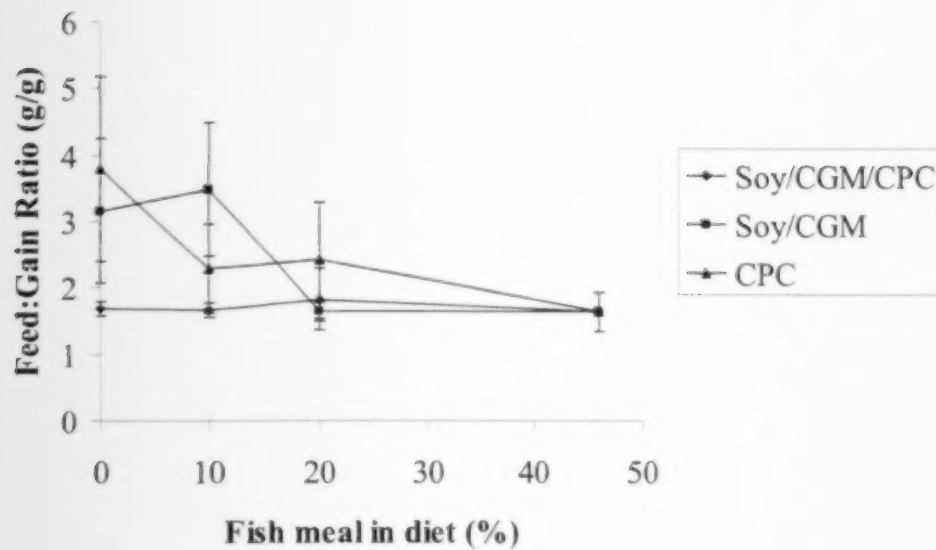
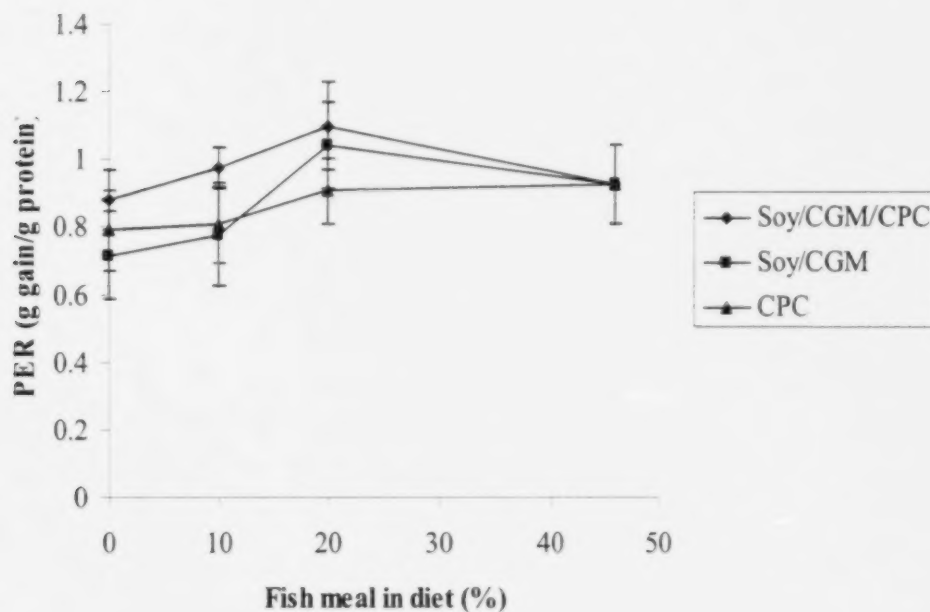


Figure 4. Protein efficiency ratios of tilapia fed diets with simple or complex mixtures of vegetable proteins replacing fish meal.



Appendix VI

Effect of adding protease to coextruded flax:pea or canola:pea products on nutrient digestibility and growth performance of rainbow trout (*Oncorhynchus mykiss*)

Submitted to Animal Feed Science and Technology

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Abstract

Limited supplies of fish meal and oil are a major constraint to the growth of the aquaculture feed industry. Coextruded flax:pea (F:P) and canola:pea (C:P) mixtures are commercially available products that have desirable fatty acid compositions but low protein digestibility. Three experiments were performed to determine if adding a protease to diets containing F:P or C:P would improve protein digestibility in rainbow trout. The addition of 250 g protease tonne⁻¹ to C:P resulted in significant increases in coefficients of total tract apparent digestibility (CTTAD) for crude protein, lipid, energy and dry matter ($P < 0.05$). However, when the protease was added to F:P, there was no effect on the CTTAD of crude protein, lipid, energy or dry matter. In a second experiment, the effect of adding protease to diets containing either L:P or C:P on the growth performance of rainbow trout was assessed. The fish were fed one of five diets including: 1) fish meal control, 2) 240 kg tonne⁻¹ L:P, 3) Diet 2 plus 250 g tonne⁻¹ of protease, 4) 240 kg tonne⁻¹ C:P, 5) Diet 4 plus 250 g tonne⁻¹ of protease. The diets were balanced for digestible protein, lipid and energy and fed to the fish over an eight week period. The fish fed C:P or L:P had reduced weight gain compared to the controls but there were no significant differences in specific growth rates ($P < 0.05$). The addition of protease to the C:P diet resulted in a significant improvement in feed efficiency. However, the addition of protease to the F:P diet had no effect on growth performance. To assess whether the mucilage content of the flax in L:P was responsible for the lack of effect of protease, the digestibility of crude protein, lipid, energy and dry matter in dehulled flaxseed with and without 250 g protease tonne⁻¹ was measured. The addition of protease had no effect on the digestibility of dehulled flaxseed in rainbow trout suggesting that the mucilage

content of flax does not inhibit the activity of this protease. The use of a commercial protease improved the nutrient digestibility of C:P but not F:P in rainbow trout. The benefits of protease addition should therefore be assessed for individual ingredients.

Keywords: rainbow trout, flax, canola, peas, protease, digestibility

Abbreviations: Coextruded flax:pea, L:P; Coextruded canola:pea, C:P; Coefficient of total tract apparent digestibility, CTTAD; DM, dry matter.

Introduction

With the aquaculture feed industry expected to triple production in the next 10 years (New, 1999), limited supplies of fish meal and fish oil threaten to constrain this expansion. The challenges of creating new plant-based feed ingredients for salmonid diets are 1) providing high quality protein and 2) providing a source of n-3 fatty acids. In the last decade, a large body of research has been performed on the nutrient properties of various plant-source proteins in salmonid fish including soya bean meal, canola/rapeseed meal, peas, lupins and flax (Gomes et al., 1993; Gomes et al., 1995; Pfeffer et al., 1995; Adelizi et al., 1998; Arndt et al., 1999; Mambrini et al., 1999; Mwachireya et al., 1999; Burel et al., 2000; Carter and Hauler, 2000; Thiessen et al., 2003a). The digestibility of crude protein in these plant sources is generally lower than that of fish meal in salmonids. Attempts to improve the digestibility of plant proteins have included the use of hydrothermal treatments such as extrusion, fractionation of crops to reduce the content of antinutritional factors and increase the protein concentration (Thiessen et al., 2003a; 2003b) and the use of low inclusion rates of a number of plant protein sources to reduce exposure to individual antinutritional factors (Gomes et al., 1995; Adelizi et al., 1998). While each of these approaches have been shown to improve protein and energy digestibility of plant protein sources, the digestibility of many ingredients remains low compared to fishmeal.

Several studies have investigated the use of flax and canola as feed ingredients for rainbow trout (Thiessen et al., 2003a; 2003b). Coextrusion of whole flax and full-fat canola with peas in a 1:1 ratio results in products with high lipid digestibility and acceptable handling properties. The oil fractions of these products have high CTTAD

when fed to rainbow trout (0.845 and 0.935 for F:P and C:P respectively) (Drew, unpublished data). However, the CTTAD of crude protein for these products is relatively low (0.781 and 0.764 for L:P and C:P respectively) (Drew, unpublished data).

The use of proteases to improve protein digestibility has been researched extensively in poultry and pigs. Studies in pigs have reported significantly improved protein digestibility and feed efficiency (O'Doherty and Forde, 1999). In broiler chickens, the addition of protease to soya bean meal resulted in a significant improvement in true nitrogen digestibility (Ghazi et al., 2003). However, there are no published reports on the effect of adding proteases to salmonid diets on digestibility of feed proteins or fish growth performance. The following studies were therefore conducted to examine the effect of a commercial protease widely used in poultry diets on the digestibility coextruded flax:pea and canola:pea products in rainbow trout.

Materials and Methods

Experiment 1

The fish used in all experiments were maintained in accordance with the guidelines of the Canadian Council on Animal Care (Canadian Council on Animal Care, 1993). Rainbow trout (rainbow/steelhead strain) were acquired from Cangro Processors Ltd. (Lucky Lake, SK, Canada). The fish were housed in 350 l tanks that were part of a recirculating system using biological filtration. Water temperature was maintained at $15 \pm 1^\circ \text{C}$. Dissolved oxygen, nitrate, nitrite, ammonia and pH were monitored daily and photoperiod used was a 14 h light/10 h dark cycle. Seventeen (276.5g; average weight) fish per tank were used with five tanks per treatment. The L:P and C:P were 1:1 mixtures of whole flax seed with field pea and full fat canola seed with field pea respectively and were produced by Oleet

Processing (Regina, SK). The two products were processed by coextrusion (Insta-Pro Model 600 extruder, Insta-Pro International, Des Moines IA) at a temperature of 140°C. The CTTADs were measured using an indirect method with diets containing 10 g kg⁻¹ Celite™ (Celite Corporation, Lompoc, California, USA) as a nonabsorbable indicator. A reference diet (Table 1) was formulated according to Bureau and Cho (1994) and the experimental diets were formulated using 700 g kg⁻¹ of the reference diet with 300 g kg⁻¹ of F:P or C:P (dry matter basis). The commercial protease used (Poultrygrow-250™; JEFO Nutrition Inc., St Hyacinthe QC) is a proprietary bacterial fermentation product. The protease is an alkaline serine endopeptidase with a pH optimum of 8.55 and an activity of 5.84 USP units per mg product on an as is basis. There are no known secondary enzyme activities. The protease was added to separate reference and experimental diets at a rate of 250 g tonne⁻¹. The diets were cold extruded in a pasta maker, dried in a forced air oven (55°C, 12 h), chopped and screened to obtain the appropriate pellet size. The fish were adapted to the experimental diets for five days and fecal material collected over a seven day period using a settling column which separated the fecal material from the effluent water. Following collection, feces were centrifuged (5000 X g, 15 min), frozen and freeze dried for analysis.

Experiment 2

Rainbow trout (same strain and source as Experiment 1) were maintained in 75 l tanks that were part of a recirculating system using biological filtration. Water temperature was maintained at 15 ± 1 °C. Dissolved oxygen, nitrate, nitrite, ammonia and pH were monitored daily and the photoperiod used was a 14 h light/10 h dark cycle. A total of three hundred rainbow trout weighing 190.0 g on average were randomly assigned to 30

tanks (10 fish per tank) and six tanks each were randomly assigned to one of 5 experimental diets: 1) Fishmeal control, 2) 240 kg tonne⁻¹ F:P, 3) Diet 2 plus 250 g tonne⁻¹ of protease, 4) 240 kg tonne⁻¹ C:P and 5) Diet 4 plus 250 g tonne⁻¹ of protease. The control diet was based on fish meal (Table 4). All diets contained the same levels of corn gluten meal and blood meal. Fish meal, fish oil and wheat were replaced with F:P or C:P to give diets with equal digestible protein, lipid and energy contents using the CCTAD from Experiment 1 (Table 5). The diets were manufactured as described above. The fish were fed by hand to satiation twice daily during the 56 day experimental period. The total tank weight of the fish was recorded on days 0, 28 and 56 and feed intake was recorded daily. Growth performance was determined by weight gain (g fish⁻¹), specific growth rate ($[(\ln \text{ final weight} - \ln \text{ initial weight})/\text{days} \times 100]$), total feed intake (g fish⁻¹) and feed conversion ratio (feed gain⁻¹).

Experiment 3

Fish were maintained as described in Experiment 1. Ten (436.5g average weight) fish per tank were utilized with four tanks per treatment. Dehulled flaxseed was prepared by abrasive dehulling using the method of Oomah et al., (1996) (Prairie Agricultural Machinery Institute, Humboldt SK). The viscosity of the flax before and after dehulling was determined using the method of Cui et al., (1994a). Briefly, 25 g of sample was added to 325 mL of distilled water maintained at 85°C and the mixture was stirred for 3 h. The supernatant was then cooled to 22°C and its viscosity was determined using a digital Brookfield Programmable Rheometer (Model DV-111; Brookfield Engineering Inc. Stoughton, MA, USA). Readings were taken at the speed of 250 rpm using spindle RV-1.

The dehulled flaxseed was autoclaved (121°C, 20 min; 1 bar) and ground (Retsch GM200 mill; 4500 rpm for 10 sec, 6000 rpm for 10 sec) prior to inclusion into the diets. Protease was included at a rate of 250 g tonne⁻¹ in the diet. The diets were formulated and manufactured as in Experiment 1. The fish were adapted to the experimental diets for seven days and fecal material collected over a five day period using a settling column which separated the fecal material from the effluent water. Following collection, feces were centrifuged (5000 x g, 15 min), frozen and freeze dried for analysis. The coefficients of total tract apparent digestibility (CTTAD) for the reference and experimental diets were calculated as follows (Sugiura et al. 1998):

Laboratory Analysis

Experimental diets, ingredients and fecal material were analyzed for moisture (AOAC 1990, method no. 934.01), energy (oxygen bomb calorimetry; Parr Adiabatic Calorimeter, Model 1200), and acid ether extract (AOAC, 1995, method no. 954.02). Nitrogen content samples were analysed for N by combustion method (method 968.06; AOAC 1990) using a Leco protein/N determinator (model FP-528, Leco Corporation, St Joseph, MI). Protein was estimated by multiplying nitrogen content by 6.25. Acid insoluble ash was analyzed using the method of Vogtmann et al. (1975).

Statistical Analysis

The results were analyzed by analysis of variance using the GLM procedure of SAS (SAS Institute, 1989). Mean values were separated using the Student-Newman-Keuls test and considered significant when $P < 0.05$.

Results

Experiment 1

The nutrient composition of the ingredients and experimental diets used in this experiment are shown in Tables 2 and 3, respectively. The apparent dry matter, crude protein, acid ether extract and energy digestibility of the experimental ingredients are reported in Table 4.

The crude protein CTTAD of the F:P was 0.849. The addition of protease to the F:P diet had no significant effect on the crude protein CTTAD of the F:P which actually decreased to 83.6%. The crude protein CTTAD of the C:P (0.760) was significantly lower than for F:P ($P < 0.05$). The addition of protease to the F:P diet did not significantly affect the CTTAD of acid ether extract, gross energy or dry matter. In contrast, the addition of protease to the C:P ingredient resulted in a significant increase in crude protein CTTAD (0.720 vs. 0.836; $P < 0.05$). Furthermore, the CTTAD of acid ether extract, energy and dry matter were also significantly improved by the addition of protease to the C:P diet but not the F:P diet. The acid ether extract CTTAD of F:P was significantly lower than for C:P ingredient (0.843 vs. 0.899). There were no significant differences in the energy and dry matter CTTAD's of F:P and C:P.

Experiment 2

The feed intake, weight gain, feed conversion ratio, and specific growth rate of the fish for the eight week experimental period are shown in Table 6. Feed intake by fish fed the C:P + protease diet was significantly lower ($P < 0.05$) than intake of the other four dietary treatments throughout the eight week experiment. However, the weight gains of the fish fed the C:P + protease diet were not significantly different from those of the fish

fed the Control, F:P + protease or C:P diets during the 0-4 week period or those fed the F:P, F:P + protease or C:P diets during the 0-8 week period. Furthermore the feed conversion ratios of fish fed the C:P + protease diet were significantly higher than those fed the C:P diet ($P < 0.05$). Although not significant, the addition of protease to the F:P diet also resulted in a numerical improvement in feed conversion ratios compared to fish fed F:P alone. Feed conversion ratios were highest for fish fed the C:P diet. No significant differences in specific growth rate were observed between diets during the experiment.

Experiment 3

The ingredient and experimental diet nutrient composition are reported in Table 7. The dehulled flax had a viscosity of 35.2 centiPoise compared to 55.0 cP before dehulling. The apparent dry matter, crude protein, acid ether extract and energy digestibility of the experimental ingredients are reported in Table 8. CTTAD's for crude protein, acid ether extract, gross energy and dry matter were lower than those observed in Experiment 1. Furthermore, the addition of protease to the dehulled flax diet did not significantly affect the CTTADs of any of the feed fractions.

Discussion

The coextruded products used in this trial are of interest as aquafeed ingredients primarily because of their lipid content. Fish oil is the most widely used source of n-3 fatty acids which are required by salmonids to maximize growth potential and maintain the n-3 fatty acid content of the fish carcass desired by consumers. Rainbow trout can elongate and desaturate 18:3n-3 into 20:5n-3 and 22:6n-3 (Owen et al., 1975), but most plant oils are poor sources of 18:3n-3 (NRC, 1993). Exceptions to this are linseed oil and canola oil.

The C:P used in this trial was 226 g kg⁻¹ ether extract primarily from full-fat canola seed. Canola oil contains approximately 120 g kg⁻¹ 18:3n-3 as a percentage of total oil (NRC, 1993). F:P contained 255 g kg⁻¹ ether extract from linseed oil of 18:3n-3 is 533 g kg⁻¹ of total oil content (NRC, 1993). Despite the desirable composition of the oil in both C:P and F:P, the use of these ingredients to replace fish oil in salmonid diets is limited by the relatively low digestibility of their crude protein, energy, and dry matter fractions (Thiessen et al., 2003a). Several studies have reported that extrusion of full-fat canola seed with field pea improves the digestibility of protein, dry matter and energy in salmonid fish and poultry (Melcion et al., 1988; Gomes et al., 1993, 1995). However, Thacker et al., (2002) reported that coextrusion of full-fat canola seed with field pea did not improve digestibility of dry matter, crude protein or energy in diets fed to growing/finishing pigs. No studies on the digestibility of flax seed coextruded with pea have been reported.

The protein CTTAD of the C:P in this trial was only 0.760 which is lower than that reported by Gomes et al., (1995) for a similar coextruded blend of full-fat rapeseed and pea (Colzapro; protein CTTAD 0.945). The CTTAD of crude protein in commercial canola meal fed to rainbow trout has been reported to range from 0.832 (Hilton and Slinger, 1986) to 0.881 (Mwachireya et al., 1999) and the CTTAD of crude protein in pea has been reported to range from 0.804 (Gomes et al, 1995) to 0.909 (Thiessen et al., 2003a). Since the protein digestibility of the C:P used in this trial is lower than the reported protein digestibilities for either pea or canola separately, this suggests the C:P used in this trial may have been subjected to excessive heat during processing, thus reducing its protein digestibility.

The CTTAD of crude protein in the coextruded flax:pea product was significantly higher than that of the canola:pea product (0.849 vs 0.760). While there are no other published reports on the digestibility of similar coextruded flax:pea products, we have determined the CTTAD of crude protein in rainbow trout for untreated flax (0.675) and flax autoclaved for 20 minutes at 121°C; 1 bar (0.777) (Drew, unpublished data). The protein digestibility of the F:P is therefore intermediate between the digestibility of autoclaved flax and pea suggesting there was no heat damage to protein during extrusion processing.

Although there are no reports on the effects of adding protease to diets fed to salmonid fish, a number studies on the use of proteases to improve the digestibility of feed ingredients in pigs and poultry have been published. In growing-finishing pigs, O'Doherty and Forde (1999) did not observe any effect on protein or energy digestibility but did report improved feed efficiency. In broiler chickens, the addition of protease to lupin- (Rubio et al., 2003) or soya bean-based (Ghazi et al., 2003; Ghazi et al., 2002; Marsman et al, 1997) diets resulted in improved digestibility of protein. However, Szczurek et al., (2001) reported no effect of protease alone on protein or energy digestibility. The variability of these results may be due to the differences in the proteases and diet formulations used in these studies.

The addition of the protease to C:P-based diets resulted in significant improvements in the CTTADs of crude protein, energy, lipid and dry matter ($P < 0.05$) in rainbow trout. Furthermore, when the fish were fed a diet containing 240 g kg⁻¹ C:P replacing fish meal and oil, there was a significant decrease in weight gain and feed efficiency over the 8 week experimental period. The addition of protease to the C:P

improved feed efficiency but had no effect on overall weight gain. Taken together, this suggests better nutrient utilization by the fish with the addition of protease to the C:P diet.

In contrast, the inclusion of protease had no effect on the protein CTTAD of F:P or on the growth performance of the rainbow trout fed 240 g kg⁻¹ F:P replacing fish meal and oil. Flax contains antinutritional factors which may have interfered with the activity of protease including a pyridoxine antagonist (linatine), cyanogenic glycosides and mucilage (Oomah et al, 1992; Bhatti, 1993). While Linatine and cyanogenic glycosides are destroyed by heat processing, mucilage is heat stable and must be physically removed from flax to eliminate it. Mucilage is a heterogenic water-soluble carbohydrate which can increase the viscosity of digesta in the small intestine and lead to reduced nutrient availability (Bhatti, 1993). This may have been responsible for the lack of response to protease addition to the F:P diets. To test this hypothesis we determined the effect of protease added to flax with the mucilage removed.

Whole flaxseed contains approximately 80 g kg⁻¹ mucilage in the hull of the seed (Bhatti and Cherdkiatgumachai, 1990). Removal of mucilage may be accomplished by extraction with hot water or salt solutions (Cui et al., 1994b) or by dehulling the flax seed (Oomah et al., 1997). In the present study, dehulling of flax reduced its viscosity from 55.0 to 35.2 centiPoise, however, this did not result in increased CTTAD of crude protein when protease was added to the diet.

The dehulled flax was available in only limited quantities and it was not possible to extrude these small batches. The flax was ground and autoclaved to simulate the effects of extrusion. However, the CTTAD of crude protein, energy, acid ether extract and dry matter of the dehulled flax were all considerably lower than those observed for

the F:P fed in Experiment 1. This suggests that extrusion of flax results in a product with significantly improved nutrient digestibility compared to a heat treatment and grinding alone.

In conclusion, whole canola and flax seed are excellent sources of n-3 fatty acids for aquaculture feeds and the use of a commercial protease was shown to increase the nutrient digestibility of C:P. However, the same protease had no effect on the F:P product and the efficacy of proteases used in salmonid diets should therefore be assessed for individual ingredients.

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Table 1. Composition of reference diet used in Experiments 1 and 3

Ingredient	Inclusion (g kg ⁻¹)
Fishmeal ^a	300
Soya bean meal	170
Maize gluten meal	130
Wheat flour	280
Vitamin Mineral premix ^b	10
Fishoil ^d	100
Celite ^c	10
(Total)	1000

^aSouth American aquagrade; EWOS Canada Ltd.

^b Vitamin Mineral premix (mg kg⁻¹ dry diet unless otherwise stated): vitamin A (as acetate), 7500 IU kg⁻¹ dry diet; vitamin D3 (as cholecalciferol), 6000 IU kg⁻¹ dry diet; vitamin E (as dl- α -tocopheryl-acetate), 150 IU kg⁻¹ dry diet; Vitamin K (as menadione Na-bisulfate) 3; vitamin B12 (as cyanocobalamin), 0.06; Ascorbic acid (as ascorbyl polyphosphate), 150; d-biotin, 42; choline (as chloride), 3000; folic acid, 3; niacin (as nicotinic acid), 30; pantothenic acid, 60; pyridoxine, 15; riboflavin, 18; thiamin, 3; NaCl, 6.15; ferrous sulfate, 0.13; copper sulfate, 0.06; manganese sulfate, 0.18; potassium iodide, 0.02; zinc sulfate, 0.3; carrier (wheat middling or starch)

^cCelite 545, <125 μ m; Celite Corporation, World Minerals Co., Lompoc, CA, USA

^dMixed variety fish oil; EWOS Canada Ltd.

Table 2. Analysis of experimental ingredients used in Experiment 1

	F:P	C:P
Dry matter (g kg ⁻¹)	925	917
Gross energy (MJ kg ⁻¹) ^a	23.5	23.1
Crude protein (g kg ⁻¹) ^a	233	262
Ether extract(g kg ⁻¹) ^a	255	226

^aDry matter basis

Table 3. Analysis of the experimental diets used in Experiment 1

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
	(F:P)	(C:P)	(F:P + Protease)	(C:P+ Protease)	(Reference)	(Reference + Protease)
Dry matter ^a	948	952	958	958	950	953
Gross energy ^b	22.5	22.4	22.6	22.6	22.1	22.1
Crude protein ^a	377	387	382	389	444	450
Acid ether extract ^a	187	168	188	179	160	161
Acid insoluble ash ^a	8.5	10.0	9.9	9.0	11.3	13.2

^aValues g kg⁻¹ dry matter basis

^bValues are MJ kg⁻¹ dry matter basis

Table 4. Coefficients of total tract apparent digestibility of L:P, C:P, with and without protease in rainbow trout (n=5)

	F:P	C:P	F:P + Protease	C:P + Protease	SEM
Dry matter	0.549 ^b	0.625 ^b	0.554 ^b	0.813 ^a	2.76
Gross energy	0.615 ^b	0.681 ^b	0.618 ^b	0.831 ^a	2.24
Crude protein	0.849 ^a	0.760 ^b	0.836 ^a	0.836 ^a	0.99
Acid ether extract	0.843 ^c	0.899 ^b	0.853 ^c	0.968 ^a	1.23

^{a-d}Means with different superscripts within a row are significantly different ($P < 0.05$).

Table 5. Formulation of diets used in Experiment 2 (kg tonne⁻¹)

Ingredient	Control	F:P +		C:P +	
		F:P	protease	C:P	protease
Fishmeal	425	365	365	355	355
Maize gluten meal	120	120	120	120	120
Wheat flour	190	130	130	130	130
L:P	0	240	240	0	0
C:P	0	0	0	240	240
Blood meal	50	50	50	50	50
Fish oil	122	70	70	68	68
Choline	5	5	5	5	5
Vitamin premix ^a	2.5	2.5	2.5	2.5	2.5
Mineral premix ^b	2.5	2.5	2.5	2.5	2.5
Alpha cellulose	83	15	14.75	27	26.75
Protease	0	0	0.25	0	0.25
TOTAL	1000	1000	1000	1000	1000

^aVitamin premix (mg kg⁻¹ dry diet): vitamin A (as acetate), 7500 IU kg⁻¹ dry diet; vitamin D3 (as cholecalciferol), 6000 IU kg⁻¹ dry diet; vitamin E (as dl- α -tocopheryl-acetate), 150 IU kg⁻¹ dry diet; Vitamin K (as menadione Na-bisulfate) 3; vitamin B12 (as cyanocobalamin), 0.06; Ascorbic acid (as ascorbyl polyphosphate), 150; d-biotin, 42; choline (as chloride), 3000; folic acid, 3; niacin (as nicotinic acid), 30; pantothenic acid, 60; pyridoxine, 15; riboflavin, 18; thiamin, 3; carrier (wheat middling or starch)

^bMineral premix (mg kg⁻¹ dry diet): NaCl, 6.15; ferrous sulfate, 0.13; copper sulfate, 0.06; manganese sulfate, 0.18; potassium iodide, 0.02; zinc sulfate, 0.3; carrier (wheat middling or starch)

Table 6. The feed intake, weight gain, feed conversion ratio and specific growth rate of rainbow trout fed L:P and C:P with or without protease

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM ¹
	(Control)	(F:P)	(F:P + protease)	(C:P)	(C:P + protease)	
Feed intake (g fish⁻¹)						
<i>0-4 weeks</i>	84.2 ^a	88.4 ^a	80.1 ^a	82.3 ^a	69.3 ^b	3.63
<i>0-8 weeks</i>	212.6 ^a	203.5 ^a	189.8 ^{ab}	198.2 ^{ab}	174.0 ^b	7.26
Weight gain (g fish⁻¹)						
<i>0-4 weeks</i>	86.6 ^{ab}	90.8 ^a	86.4 ^{ab}	78.6 ^{ab}	72.0 ^b	4.21
<i>0-8 weeks</i>	197.0 ^a	179.7 ^{ab}	171.6 ^b	164.2 ^b	155.7 ^b	6.52
Feed Conversion Ratio (g feed g gain⁻¹)						
<i>0-4 weeks</i>	0.98 ^{ab}	0.98 ^{ab}	0.93 ^b	1.05 ^a	0.96 ^b	0.0215
<i>0-8 weeks</i>	1.08 ^b	1.13 ^b	1.11 ^b	1.21 ^a	1.12 ^b	0.0240
Specific Growth Rate (%)						
<i>0-4 weeks</i>	1.39 ^a	1.23 ^a	1.33 ^a	1.27 ^a	1.33 ^a	0.0751
<i>0-8 weeks</i>	1.19 ^a	1.13 ^a	1.21 ^a	1.18 ^a	1.13 ^a	0.0532

¹SEM denotes standard error of the mean.

^{a,b}Means with different superscripts within a row are significantly different ($P < 0.05$).

Table 7. Analysis of the dehulled flax and diets used in Experiment 3

	Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
	Dehulled	(Dehulled	(Dehulled flax	(Reference)	(Reference
	flax	flax)	+ protease)		+ protease)
Dry matter ^a	923	0.957	0.955	0.938	94.9
Gross energy ^b	28.0	24.3	24.3	22.4	22.3
Crude protein ^a	245	393	392	461	458
Acid ether extract ^a	328	231	239	171	170
Acid insoluble ash ^a	-	9.0	8.8	12.6	12.7

^aValues g kg⁻¹ dry matter basis

^bValues are MJ kg⁻¹ dry matter basis

Table 8. Coefficients of total tract apparent digestibility of dehulled flaxseed and dehulled flaxseed with protease in rainbow trout (n=4)

	Dehulled flax	Dehulled flax + protease	SEM
Dry matter	0.554 ^a	0.518 ^a	2.16
Gross energy	0.496 ^a	0.477 ^a	2.09
Crude protein	0.631 ^a	0.616 ^a	1.54
Acid ether extract	0.464 ^a	0.468 ^a	2.07

^aNo significant differences in treatment means were observed ($P > 0.05$).

Appendix VII

Replacement of fish meal by canola protein concentrate in diets fed to rainbow trout

(*Oncorhynchus mykiss*)

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Running title: Canola protein concentrate for rainbow trout

Key words: Canola protein concentrate; anti nutritional factors; rainbow trout; digestibility; performance

Abstract

A novel protein concentrate (CanPro-IP65) prepared from canola seed was assessed for nutrient digestibility and performance in rainbow trout (*Oncorhynchus mykiss*). CanPro-IP65 contains 72.4% crude protein, 4.8% arginine, 3.5% lysine, 1.5% methionine, 3.0% threonine and no detectable phytic acid. The apparent digestibility coefficients were: dry matter, 81.7%; crude protein, 89.9%; gross energy, 86.1%; arginine, 94.5%; lysine, 93.5%; methionine, 95.4%; threonine, 89.3%. In a 9-week performance trial, fish were fed a fish meal control diet, and test diets consisting of CanPro-IP65 replacing 50 and 75% of fish meal protein; water-washed CanPro-IP65 replacing 50 and 75% of fish meal protein; and CanPro-IP65 plus an attractant replacing 50 and 75% of fish meal protein. No significant differences in performance were observed ($P > 0.05$). The addition of an attractant reduced a trend toward decreased performance at the 75% fish meal protein replacement level. In a subsequent 9-week performance trial, CanPro-IP65 was incorporated into commercial-like trout diets at 10%, 20% and 30% replacement of fish meal protein. No significant differences in performance were obtained ($P > 0.05$). These studies show that CanPro-IP65 has potential to replace substantial levels of fish meal in diets for carnivorous fish without compromising performance.

Introduction

Canola is the name given to selected varieties of rapeseed that are low in glucosinolate (<30 μ moles of alkenyl glucosinolates per gram of oil-free dry matter of seed) and erucic acid (<2% of total fatty acids in the oil) (Bell 1993). Canola meal, resulting from the solvent extraction of canola oil, is readily available worldwide. Relative to the nutrient requirements of carnivorous fish such as Atlantic salmon and rainbow trout, the amino acid profile of canola meal protein is similar to that of herring meal protein and superior to soybean meal protein (Higgs et al. 1995; Mwachireya et al. 1999).

As with most plant protein sources, canola meal contains antinutritional factors that may restrict growth performance and protein utilization of salmonid fish. The major antinutritional factors include fibre, oligosaccharides, phenolic compounds, phytic acid and glucosinolates (Bell 1993, Higgs et al. 1995). Indigestible carbohydrates in canola meal [crude fibre (10.6%), cellulose (14.5%), hemicellulose (5.0%) and lignin (8.3%); Mwachireya et al. 1999] can not be used by salmonid fish and can diminish the nutritional value of other dietary ingredients. The phytic acid content of canola meal ranges from 3.1 – 3.7% (Higgs et al. 1995) and significantly reduces phosphorus digestibility, protein utilization and growth (Spinelli et al. 1983, Forster et al. 1999). Furthermore, an increased incidence of cataracts and structural changes in the pyloric ceca of rainbow trout have been attributed to elevated levels of phytic acid (Higgs et al. 1995). While glucosinolate levels are considerably reduced in contemporary commercial varieties of canola, there is still concern over the effect this factor has on thyroid function, feed acceptance, liver and kidney function and growth performance in fish

(Hardy and Sullivan 1983, Higgs et al. 1983, Hilton and Slinger 1986, Leatherland and Hilton 1988, McCurdy and March 1992).

The nutritional value of canola meal in aquaculture diets may be significantly improved by fractionating and then concentrating the protein component of the meal (Higgs et al. 1994, Mwachireya et al. 1999). Apparent nutrient digestibility of canola protein concentrate and isolate in rainbow trout were significantly increased compared to canola meal (Higgs et al. 1994, Mwachireya et al. 1999). In addition, an increase in the level of canola protein concentrate in the diet, with a relative decrease in the level of fish meal, led to an increase in protein digestibility of the diet, suggesting that canola protein was more digestible than fish meal protein (Stickney et al. 1996, Forster et al. 1999). Inclusion of an attractant into an enzyme dephytinized rapeseed protein concentrate diet allowed for replacement of 100% of the dietary protein derived from fish meal without a reduction in performance of trout (Higgs et al. 1995).

CanPro-IP65 is a novel canola protein concentrate that is enriched in insoluble protein and devoid of measurable levels of phytic acid. The objective of the present study was to assess the nutrient digestibility of CanPro-IP65 in juvenile rainbow trout and to assess growth and nutrient utilization in trout fed increasing levels of CanPro-IP65 as a substitute for fish meal and high fishmeal diets and commercial-like diets containing a mixture of fishmeal, corn gluten meal and soybean meal.

Materials and methods

Fish Management

Rainbow trout (rainbow/steelhead strain) were acquired from Cangro Processors Ltd. (Lucky Lake, SK, Canada). The fish were maintained in 120-L tanks that were part of an

indoor, semi-closed recirculating system utilizing biological filtration. Water temperature was maintained at $14 \pm 1^\circ\text{C}$. Photoperiod was a 14-h light:10-h dark cycle. The digestibility experiment used a total of 400 rainbow trout weighing 106 ± 9 g (average weight) randomly assigned to 16 tanks (25 fish per tank). Four replicates were used for each diet. The first growth experiment utilized a total of 500 rainbow trout weighing 28 ± 3 g randomly assigned to 20 tanks (25 fish per tank). Four replicates were used for each diet. The second growth experiment used a total of 250 rainbow trout weighing 179 ± 15 g randomly assigned to 25 tanks (10 fish per tank). Five replicates were used for each diet. In all experiments, the fish were fed by hand to apparent satiation twice daily. The fish were maintained in accordance with the guidelines of the Canadian Council on Animal Care (CCAC 1984).

Diet Preparation

The digestibility experiment evaluated three ingredients: CanPro-IP65 provided by MCN Bioproducts Ltd. (Saskatoon, SK), corn gluten meal obtained from the University of Saskatchewan feed mill (Saskatoon, SK) and a mixed variety of South American Aquagrade fish meal (EWOS Canada Ltd., Surrey, BC, Canada). The ingredients were analyzed for energy, protein and amino acid content (Table 1). All ingredients were ground to 1.0 mm particle size (Christy & Norris Laboratory Mill, Chelmsford, England). A reference diet using 1% celite (Celite 545, $<125 \mu\text{m}$; Celite Corp., World Minerals Co., Lompoc, CA, USA, Cat. No. 15913) as the indicator, was formulated (Table 3) according to Bureau & Cho (1994). Three experimental diets were made containing 70% of the reference diet and 30% of each test ingredient. The diets were cold-extruded (3.0 mm die)

and the formed strands dried (forced air oven; 55°C, 12 h), chopped and screened to obtain an appropriate size of pellet.

Seven dietary treatments were evaluated in the first growth experiment (Table 4): a 62.5% fish meal reference diet; test diets with CanPro-IP65 replacing 50% and 75% of reference diet fish meal protein; test diets containing water-washed CanPro-IP65 at 50 and 75% fishmeal protein replacement; and test diets containing a commercial palatability enhancer at 1% of diet (Finnstim[™]; Finnsugar Bioproducts, Helsinki, Finland) plus CanPro-IP65 replacing 50% and 75% of fish meal protein. Diets were balanced for crude protein, energy, lysine and fish lipid (from fish meal and oil) (Table 4). The diets were cold extruded (3.0 mm diet) as documented earlier.

Five dietary treatments were evaluated in the second growth experiment (Table 5): a commercial-like reference diet containing 39% fishmeal, 17% corn gluten meal and 10% soybean meal; test diets with CanPro-IP65 replacing 10%, 20% and 30% of the fishmeal protein in the reference diet; and a test diet containing a commercial palatability enhancer at 1% of diet (Finnstim[™]; Finnsugar Bioproducts, Helsinki, Finland) plus CanPro-IP65 replacing 30% of fish meal protein. Diets were balanced for crude protein, energy and lysine (Table 5). The diets were cold extruded (3.0 mm diet) as documented earlier.

Digestibility measurements

The fish were adapted to the experimental diets for 7 days and feces collected over night for a fourteen day period using a settling column which separated the fecal material from the effluent water. Following collection, the feces were centrifuged (Beckman, model J2-

21, Palo Alto, CA, USA; 5000 x g, 20 min), frozen and freeze-dried. Apparent digestibility coefficients were determined according to Kleiber (1961).

Growth measurements

The experimental period was 63 days for both growth experiments. The total tank weight of the fish was recorded at 21 day intervals for the duration of the experiments. Feed intake was recorded daily. Performance was determined by weight gain (g fish^{-1}), total feed intake (g 63 days^{-1}), feed conversion ratio (feed intake wet weight gain $^{-1}$), protein efficiency ratio (wet weight gain protein intake $^{-1}$) and specific growth rate ($[\ln \text{ final weight} - \ln \text{ initial weight}] \text{time}^{-1} (\text{days}) \times 100$).

Analytical methods

Phytic acid was analyzed by anion-exchange high-performance liquid chromatography with post-column detection (Rounds and Nielsen 1993). Experimental diets and fecal material were ground (Retsch mill, Brinkmann Corp.; 1.0 mm screen) prior to analysis for moisture (AOAC 1990; method no. 934.01) and energy (oxygen bomb calorimetry; Parr Adiabatic Calorimeter, Model 1200). Nitrogen content was determined by combustion method (AOAC 1995). Protein was estimated by multiplying the nitrogen content by 6.25. Amino acid analysis was determined by chromatographic analysis for which the samples were oxidized with performic acid and hydrolyzed with HCl as previously described by Llames and Fontaine (1994). The samples were analyzed for amino acids using a Beckman Amino Acid Analyzer equipped with a Spherogen 1 IEX ion exchange column, System Gold 126AA solvent delivery module, 508 auto-sampler, 166 detector, and ninhydrin 232 post column reactor, Mistral (Holland Spark, The

Netherlands). Acid ether extract analysis was performed according to AOAC (1995); method no. 954.02.

Determination of acid insoluble ash was performed according to the following procedure: Four replicates of each sample were charred (250 °C, 18 h), followed by a gradual increase in temperature to 500 °C (3 h), and then ashed (48 h). Four mL of 4 N HCl was added and the samples were heated at 120 °C for a minimum of 1h. Samples were then centrifuged (3000 x g, 10 min) followed by aspiration of the supernatant. Five mL of water was added, vortexed, centrifuged and aspirated two times. The samples were dried overnight (80 °C) followed by ashing (500 °C, 24 h).

Statistical analysis

All data were subjected to analysis of variance using the General Linear Model procedure of the Statistical Analysis System (SAS Version 8.2 2001). Differences between treatment means were determined using the Student-Newman-Keuls test at the 0.05 level of significance.

Results

Apparent digestibility of CanPro-IP65, corn gluten meal and fish meal

The crude protein digestibility for all test ingredients is shown in Table 6. The crude protein digestibility for CanPro-IP65 was 89.9% compared to 89.6% and 91.1% for fish meal and corn gluten meal respectively. Apparent individual amino acid digestibility confirmed the high crude protein digestibility results. Essential amino acid digestibility was greater than 90.0% for CanPro-IP65, corn gluten meal and fish meal with the exception of phenylalanine and threonine in CanPro-IP65, lysine in corn gluten meal and phenylalanine in fish meal. Individual essential amino acid digestibility in CanPro-IP65

were similar to, or higher than, fish meal for arginine, isoleucine, methionine, phenylalanine and valine ($P < 0.05$). Individual essential amino acid digestibility of histidine, leucine, lysine, and threonine in CanPro-IP65 was slightly lower than in fish meal ($P < 0.05$). Individual essential amino acid digestibility of CanPro-IP65 was similar to, or higher than, individual essential amino acid digestibility of corn gluten meal with the exception of leucine and phenylalanine ($P < 0.05$).

Energy digestibility (Table 6) of all experimental ingredients was very high ($>86.1\%$). A small but significant difference in energy digestibility between experimental ingredients existed ($P < 0.05$). Overall dry matter digestibility was highest for corn gluten meal and similar for CanPro-IP65 and fish meal ($P < 0.05$).

Performance of trout fed CanPro-IP65 as a replacement for dietary fish meal

Figure 1 shows the performance of rainbow trout over a 9 week period when fed a fish meal based control diet as compared to performance obtained with 50% and 75% replacement of dietary fish meal protein with CanPro-IP65 or washed CanPro-IP65, in addition to two treatments utilizing a feed attractant in the CanPro-IP65 based diets. No significant differences were obtained for feed intake, weight gain, feed conversion or protein efficiency ratio between treatment.

Figure 2 shows the performance of rainbow trout over a 9 week period when fed a typical commercial-like trout diet as compared to performance obtained with up to 30% of the dietary fish meal protein replaced with CanPro-IP65, along with a treatment utilizing a feed attractant in the highest level of inclusion of CanPro-IP65. No significant differences were obtained for feed intake, weight gain, feed conversion, specific growth rate or protein efficiency ratio between trout consuming the fish meal reference diet and

trout consuming the diets with CanPro-IP65 replacing 10%, 20% or 30% of dietary fish meal protein ($P>0.05$). By day 63 trout consuming the diet with CanPro-IP65 replacing 10% of dietary fish meal protein had a significantly greater weight gain, better feed conversion, higher specific growth rate and more efficient dietary protein utilization than trout consuming the diet with CanPro-IP65 replacing 30% of dietary fish meal protein ($P<0.05$).

Discussion

In previous experiments phytic acid, glucosinolates and fibre have been implicated in reducing the nutrient value of canola products and as a cause of palatability problems (Hardy and Sullivan 1983, Hilton and Slinger 1986, Leatherland and Hilton 1988). A high content of hulls and indigestible polysaccharides in conventional canola meal may decrease dry matter digestibility and is thought to exert a greater negative influence on nutritional value than the presence of other antinutritional compounds (Hilton and Slinger 1986, Mwachireya et al. 1999).

Phytic acid (hexaphosphates of myo-inositol) is the primary phosphate reserve in oilseed, cereal and legume seeds. Phytic acid is found in close association with the protein fraction of oilseeds and, as a result, oilseed protein concentrates and isolates have an elevated phytic acid level (Thompson 1989). Due to the ability of phytic acid to complex with proteins at acidic pH and with polyvalent cations at intestinal pH, mineral, protein and starch solubility, availability and absorption may be altered (Rickard and Thompson 1997). Several early studies indicate that high dietary phytic acid levels (25.8 g kg^{-1}) may lead to increased incidence of cataracts (due to low zinc bioavailability) and structural changes in the pyloric caeca of salmonid fish (due to low magnesium

bioavailability) (Richardson et al. 1985, Richardson et al. 1986). The phytic acid present in undephytinized rapeseed protein concentrate exerted a negligible influence on protein availability in salmonid fish (Higgs et al. 1995), however, the effect of phytic acid on growth performance and weight gain in rainbow trout fed canola products has been variable (Spinelli et al. 1983, Teskeredzic et al. 1995).

Much research has focused on the negative effect glucosinolates have on growth performance in salmonid species (Yurkowski 1978, Higgs et al. 1983, Hardy and Sullivan 1983, Hilton and Slinger 1986, Leatherland and Hilton 1988, and McCurdy and March 1992). Compensatory thyroid activity to counteract impairment of thyroid hormone synthesis and conversion of thyroxine (T_4) to triiodothyronine (T_3) has been demonstrated in salmon (Higgs et al. 1983) and rainbow trout (Yurkowski 1978, Hilton and Slinger 1986, Leatherland and Hilton 1988). Trout fry appear to be more sensitive to glucosinolates (Hilton and Slinger 1986). Elevated thyroid activity in young fish likely results in a greater response to high glucosinolate levels (Hardy and Sullivan 1983). Additionally, glucosinolates may have an affect on feed acceptance if canola products are included at high levels in the diet (Higgs et al. 1983, McCurdy and March 1992, Bell 1993).

CanPro-IP65 is a concentrated protein source with high levels of arginine, methionine and threonine and moderate levels of lysine. This balance of amino acids approaches that of the highest quality animal protein sources including fish meal. CanPro-IP65 has a significantly reduced fibre component (3.0% crude fibre) relative to conventional commercial canola meal (10.6% crude fibre). Additionally, CanPro-IP65 is

devoid of measurable levels of phytic acid and contains minimal levels of glucosinolates ($3.44 \mu\text{moles g}^{-1}$).

Nutrient digestibility of CanPro-IP65 is similar to published protein, energy and dry matter digestibility data for other canola protein concentrates (Higgs et al. 1994) and canola protein isolates (91% protein) (Mwachireya et al. 1999). Individual amino acid digestibility of CanPro-IP65 was generally in the range of 90%, comparable to fish meal, suggesting negligible amino acid damage during processing.

In the present experiment CanPro-IP65 replaced up to 75% of the fish meal component (CanPro-IP65 accounted for 71% of dietary protein) of rainbow trout diets without affecting feed intake, weight gain and feed conversion. At 75% replacement a trend toward lower feed intake and lower growth was noted. This effect was reduced by the inclusion of a palatability enhancer (Finnstim™) into the diet. Previous research has shown that enzyme dephytinized rapeseed protein concentrate (59% of dietary protein), with inclusion of a palatability enhancer, can replace 100% of dietary fish meal in trout diets without compromising performance (Prendergast et al. 1994). Teskeredzic et al. (1995) indicated that up to 66% of fish meal protein in rainbow trout diets could be replaced with dephytinized rapeseed protein (with inclusion of a palatability enhancer) without impact on trout performance over an 84 day time period. Similarly, trout fed a diet comprised of canola protein concentrate (59% of dietary protein) replacing 66% of the protein provided by fish meal had comparable growth, feed efficiency and protein utilization to trout fed a commercial diet (Forster et al. 1999).

The results of the present experiment clearly show that CanPro-IP65 can substitute for high levels of fish meal in diets fed to cultured trout without affecting the

performance of the fish. Further confirmation of the comparable nutritive value of CanPro-IP65 to fish meal was obtained in the second performance experiment in which CanPro-IP65 was incorporated into typical commercial-like trout diets at levels replacing 10%-30% of fish meal protein without compromising trout growth performance.

CanPro-IP65 is a concentrated protein ingredient with an excellent amino acid balance and a low level of fibre, glucosinolates and phytic acid. Removal of antinutritional factors increases the bioavailability of nutrients thereby allowing the successful replacement of fish meal with CanPro-IP65 in trout diets. CanPro-IP65 is a protein ingredient with enhanced value that has potential for fish meal substitution in aquafeeds.

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Table 1 Nutrient analysis of CanPro-IP65, corn gluten meal and fish meal (g kg⁻¹)

	CanPro-IP65	Corn Gluten Meal	Fish Meal ²
Dry matter	956	908	918
Gross energy (kcal kg ⁻¹) ¹	5009	5601	4915
Crude protein ¹	724	695	743
<i>Essential Amino Acids (%)</i> ¹			
Arginine	4.8	2.3	3.6
Histidine	1.9	1.3	2.7
Isoleucine	3.1	2.6	2.7
Leucine	5.7	11.3	5.2
Lysine	3.5	1.0	5.5
Methionine	1.5	1.4	1.9
Phenylalanine	3.2	4.2	2.9
Threonine	3.0	2.2	2.9
Valine	3.7	2.9	3.3
<i>Non-Essential Amino Acids (%)</i> ¹			
Alanine	3.3	6.0	4.5
Aspartic acid	6.0	4.3	6.9
Cysteic acid	1.5	1.1	0.6
Glycine	3.6	1.7	4.1
Glutamic acid	13.7	15.1	9.2
Proline	4.4	6.5	2.8
Serine	2.9	3.2	2.6

¹Dry matter basis²Mixed variety of South American Aquagrade fish meal; EWOS Canada Ltd., Surrey, BC, Canada

Table 2 Phytic acid and glucosinolate content of CanPro-IP65

	CanPro-IP65
Phytic acid (%)	0.00
Glucosinolates ($\mu\text{moles g}^{-1}$)	
<i>allyl</i>	< 0.05
<i>3-butenyl</i>	1.03
<i>4-pentenyl</i>	0.3
<i>2-OH-3-butenyl</i>	2.05
<i>2-OH-4-pentenyl</i>	< 0.05
<i>methylthiobutenyl</i>	< 0.05
<i>methylthiopentenyl</i>	< 0.05
<i>3-methylindolyl</i>	< 0.05
<i>4-OH-3-methylindolyl</i>	0.06
Total glucosinolates	3.44

Table 3 Composition of reference diet utilized in digestibility study

Ingredient	Inclusion (g kg ⁻¹)
Fishmeal ¹	300
Soybean meal	170
Corn gluten meal	130
Wheat flour	280
Vit./Min. premix ²	10
Celite ³	10
Fishoil ⁴	100
(Total)	1000

¹South American Aquagrade; EWOS Canada Ltd., Surrey, BC, Canada

²The vitamin/mineral premix was a commercial premix (EWOS; closed formulation) formulated to meet the requirements of juvenile rainbow trout.

³Celite 545, <125µm; Celite Corporation, World Minerals Co., Lompoc, CA, USA

⁴Mixed variety fish oil; EWOS Canada Ltd., Surrey, BC, Canada

Table 4 Fish meal control and CanPro-IP65 experimental diets for the first growth

experiment

		CanPro-IP65		CanPro-IP65		CanPro-IP65		Water washed	Water washed
Fish meal control diet		50% ¹	CanPro-IP65 75% ¹	50% ¹	CanPro-IP65 75% ¹	CanPro-IP65 50% ¹	CanPro-IP65 75% ¹	CanPro-IP65 50% ¹	CanPro-IP65 75% ¹
				+ attractant		+ attractant			
Ingredient composition (g kg ⁻¹)									
Fishmeal ²	625	310	155	310	155	310	155		
Wheat flour	155	105	87	95	77	134	125		
CanPro-IP65	0	330	490	330	490	300	445		
Whey	70	70	70	70	70	70	70		
Attractant ³	0	0	0	10	10	0	0		
Fish oil ⁴	140	170	180	170	180	170	185		
Lysine	0	5	8	5	8	6	10		
Choline	5	5	5	5	5	5	5		
Vitamin premix ⁵	2.5	2.5	2.5	2.5	2.5	2.5	2.5		
Mineral premix ⁵	2.5	2.5	2.5	2.5	2.5	2.5	2.5		
Nutrient Analysis (g kg ⁻¹)									
Dry matter	958	966	890	956	933	934	915		
Gross energy	5571	5709	5746	5712	5753				
(kcal kg ⁻¹) ⁶						5757	5841		
Ether extract ⁶	212	215	209	210	208	214	215		
Crude protein ⁶	521	514	518	513	511	520	527		
Ash ⁶	105	91	85	91	85	84	73		

¹CanPro-IP65 replacing 50% and 75% of dietary fish meal, respectively²South American Aquagrade; EWOS Canada Ltd., Surrey, BC, Canada³Finnstim; EWOS Canada Ltd., Surrey, BC, Canada⁴Mixed variety fish oil; EWOS Canada Ltd., Surrey, BC, Canada⁵The vitamin/mineral premix was a commercial premix (EWOS; closed formulation) formulated to meet the requirements of juvenile rainbow trout⁶Dry matter basis

Table 5 Fish meal control and CanPro-IP65 experimental diets for the second growth experiment

	Fish meal control diet	CanPro-IP65 10% ¹	CanPro-IP65 20% ¹	CanPro-IP65 30% ¹	CanPro-IP65 30% ¹ + attractant
Ingredient composition (g kg ⁻¹)					
Fishmeal ²	390	330	276	219	219
Corn gluten meal	170	170	170	170	170
Wheat flour	190	188.9	177	168	158.8
CanPro-IP65	0	60	120	180	180
Soybean meal	100	100	100	100	100
Attractant ³	0	0	0	0	10
Fish oil ⁴	140	140	145	150	150
Lysine	0	1.1	2.0	3.0	2.2
Choline	5	5	5	5	5
Vitamin premix ⁵	2.5	2.5	2.5	2.5	2.5
Mineral premix ⁵	2.5	2.5	2.5	2.5	2.5
Nutrient analysis (g kg ⁻¹)					
Dry matter	970	933	958	972	966
Gross energy (kcal kg ⁻¹) ⁶	5603	5604	5656	5692	5694
Ether extract ⁶	192	186	189	188	187
Crude protein ⁶	486	493	494	493	498

¹CanPro-IP65 replacing 10%, 20%, 30% of dietary fish meal protein, respectively

²South American Aquagrade; EWOS Canada Ltd., Surrey, BC, Canada

³Finnstim; EWOS Canada Ltd., Surrey, BC, Canada

⁴Mixed variety fish oil; EWOS Canada Ltd., Surrey, BC, Canada

⁵The vitamin/mineral premix was a commercial premix (EWOS Canada Ltd., Surrey, BC, Canada; closed formulation)

formulated to meet the requirements of juvenile rainbow trout

⁶Dry matter basis

Table 6 Apparent digestibility coefficients (ADC, %) of CanPro-IP65, corn gluten meal and fish meal in rainbow trout

	CanPro - IP65	Corn Gluten Meal	Fish Meal ¹	SEM
Crude protein	89.9 ^a	91.1 ^a	89.6 ^a	0.42
<i>Essential Amino Acids</i>				
Arginine	95.4 ^a	93.2 ^b	93.2 ^b	0.44
Histidine	92.9 ^b	91.8 ^b	94.4 ^a	0.44
Isoleucine	92.1 ^a	91.7 ^a	94.1 ^a	0.62
Leucine	90.4 ^c	92.5 ^b	94.4 ^a	0.58
Lysine	93.5 ^b	89.8 ^c	96.3 ^a	0.44
Methionine	95.4 ^a	95.3 ^a	94.9 ^a	0.60
Phenylalanine	86.9 ^b	91.2 ^a	88.9 ^b	0.65
Threonine	89.3 ^b	90.2 ^b	93.2 ^a	0.56
Valine	92.8 ^{ab}	91.9 ^b	94.1 ^a	0.45
<i>Non-Essential Amino Acids</i>				
Alanine	89.1 ^b	92.5 ^a	93.5 ^a	0.48
Aspartic acid	92.4 ^a	91.0 ^a	91.0 ^a	0.46
Cysteic acid	91.7 ^a	92.0 ^a	84.0 ^b	0.51
Glycine	91.1 ^a	90.8 ^a	89.1 ^a	0.50
Glutamic acid	94.7 ^a	93.5 ^a	94.5 ^a	0.51
Proline	95.4 ^a	93.0 ^b	89.7 ^c	0.71
Serine	88.7 ^a	90.8 ^a	90.6 ^a	0.60
Gross energy	86.1 ^b	91.3 ^a	92.5 ^a	0.83
Dry matter	81.7 ^b	90.1 ^a	84.1 ^b	1.29

^{a-d} Different letters denote significant differences within a row ($P < 0.05$).

¹ Mixed variety of South American Aquagrade fish meal (EWOS Canada Ltd., Surrey, BC, Canada)

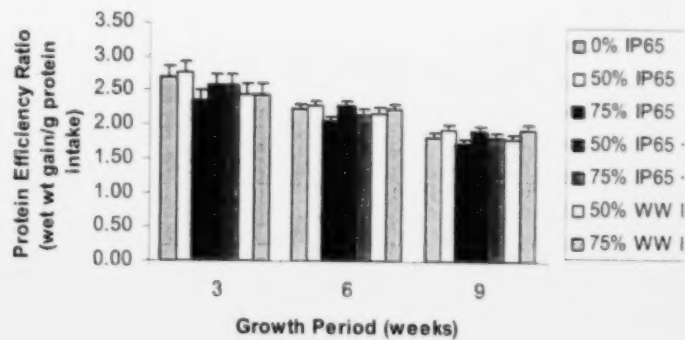
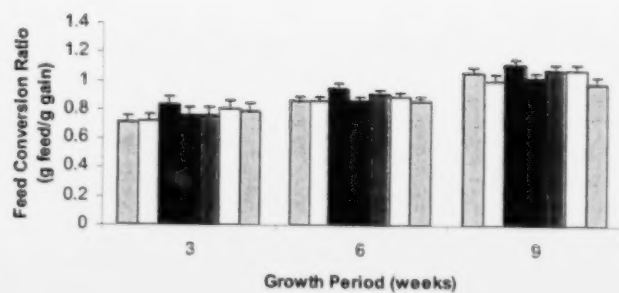
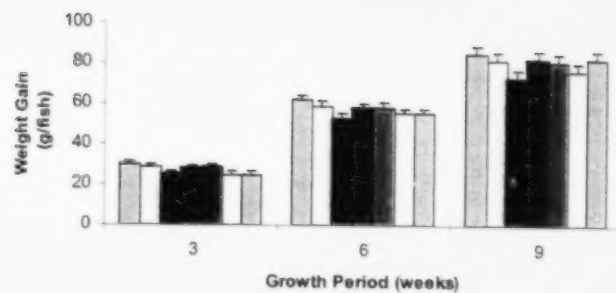
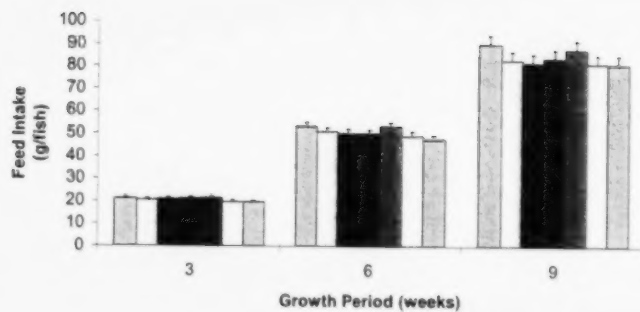


Figure 1

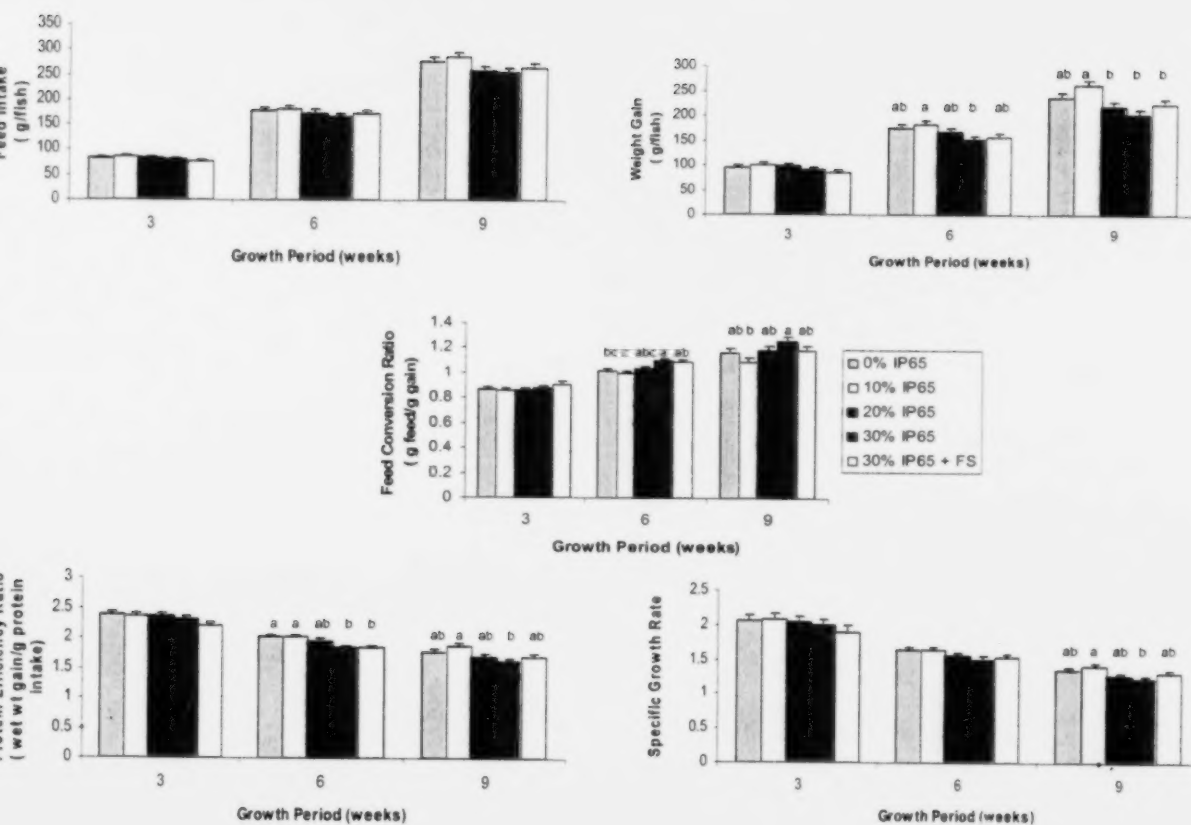


Figure 2

Figure Legend

Figure 1. The average feed intake, weight gain, feed conversion ratio and protein efficiency ratio of rainbow trout fed CanPro-IP65 as a replacement for fish meal for 63 days. Legend indicates % replacement of fish meal protein. FS indicates 1% Finstimm in the pellet, WW indicates water-washed CanPro-IP65. Means in columns without superscripts are similar ($P>0.05$).

Figure 2. The average feed intake, weight gain, feed conversion ratio, protein efficiency ratio and specific growth rate of rainbow trout fed CanPro-IP65 as a replacement for fish meal in typical trout diets for 63 days. Legend indicates % replacement of fish meal protein. FS indicates 1% Finstimm in the pellet. Means in columns without superscripts are similar ($P>0.05$).

